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A STUDY OF THE REACTION OF MESENCHYME CELLS IN THE TAD-POLE'S TAIL TOWARD INJECTED OIL GLOBULES¹

ELIOT R. CLARK

From the Anatomical Laboratory of the University of Missouri

FIVE FIGURES

In their later studies on the mode of development of the lymphatic system, Huntington and McClure have reiterated the view that the lymphatics are formed by the transformation of mesenchyme cells in the following manner. They hold that fluid accumulates in the tissue spaces, forming small lakelets; that the mesenchyme cells are pressed upon by the fluid collected; and that, as a result of this mechanical pressure, the mesenchyme cells react by flattening out and by forming membranes around the blisters.

The main evidence presented in favor of this view consists in the finding, in microscopic sections, of clear spaces in the tissues, unsurrounded by any membrane, of other spaces which have the appearance of being partly surrounded, and of others with a complete covering. Some of these appear to be completely isolated while others are connected with one another. The numerous possibilities of error in the interpretation of the appearances described have been pointed out by Miss Sabin (1), E. L. Clark (2), and myself (3), and will not be reviewed at this time.

The importance laid by these investigators on the part played by the mechanical action of fluid on mesenchyme cells may be

¹ A preliminary report of these studies was published in the Proceedings of the American Ass. of Anat., Anat. Rec., vol. 10, no. 3, 1916, p. 191.

seen from the following quotation. Huntington (4) says (page 289):

If two embryonal mesenchymal cells are separated from each other by the accumulation of fluid in the resulting intercellular space, then the opposing aspects of the two cells involved will be subjected to the mechanical and hydrostatic influences of accumulated intercellular fluid, which will react upon the surfaces of the cell still held in syncytial relation to the surrounding mesenchyme. The cells whose opposing surfaces have become freed by the development of an intercellular space, and are subjected to fluid pressure, will react as a whole, become flattened, and be transformed into endothelial cells, forming the parietal limit of an originally intercellular mesenchymal space, which is the font and origin of all vertebrate vascular development.

This should be supplemented by a quotation from McClure (5), for no explanation is given here for the accumulation of fluid in the tissues.

As soon, however, as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo and, as we know, is subsequently collected by a set of newly formed vessels, the lymphatics, which convey it to the venous circulation.

Those who maintain that the lymphatics sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of close and hollow outgrowths from the veins, the lymphatics, before it can be received into any portion of the lymphatic system.

The combined picture, then, is as follows: as soon as the blood-vessels function, "lymph begins to collect in the intercellular spaces." It collects apparently because it cannot get back into the blood-vessels. The mesenchyme cells are subjected to "mechanical and hydrostatic pressure" exerted by the accumulated lymph, and respond by flattening out, and becoming endothelial cells. This comprises the "font and origin of all vertebrate vascular development."

Some of the theoretical objections to this series of assumptions may be pointed out.

First, McClure states that "as soon as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo;" to the pressure exerted by this accumulated lymph is assigned the rôle of acting as the formative

stimulus for the transformation of mesenchyme cells into lymphatics. In another place, McClure argues that blood-vessels and lymphatics differentiate in the same manner. Now if the collection of intercellular lymph begins only after the blood-vessels begin to function, how are we to explain the collections of fluid which are supposed to have served as a formative stimulus for the differentiation of blood-vessels? Again, if both blood-vessels and lymphatics are determined solely by the action of mechanical pressure, we are left with the same puzzling problem which confronted Goette (6) in 1873, namely, why the two sets of vessels do not everywhere form communications with one another. The puzzle differs only in that Goette conceived of the circulation from blood to lymph capillary as being intracellular, and tried to explain why the same mesenchyme cell was not sometimes demanded by both the blood-vessel and the lymphatic, while Huntington and McClure conceive of the fluid as being extra-cellular and must explain why it does not happen that the same lakelet does not connect with a blood capillary on the one side, and with a lymphatic capillary on the other. Goette's puzzle has been eliminated by the proof that in the tail of amphibian larvae, mesenchyme cells take no part in the growth of blood or lymphatic vessels.

Again, it is hardly necessary that "those who maintain that the lymphatic sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of closed and hollow out-growths from the veins, the lymphatics," since it was demonstrated clearly by Magendie, over a hundred years ago, and has been proven so many times since that it cannot be questioned, that absorption of substances may take place through the blood-vessels as well as through the lymphatics.

Another assumption which is quite unwarranted on the basis of facts is that with an increase in intercellular fluid, this fluid will collect in definite lakelets, in the intercellular spaces. All our knowledge of intercellular spaces indicates that they form an irregular, intercommunicating net-work of spaces filled with fluid. It would be expected that, if this fluid were

increased, the increase in the intercellular spaces would be a general one, the distention, or edema, being regulated in its intensity only by the relative amount of resistance offered by the tissue. This resistance would, of course, be greater in dense tissues, in which the cells are bound firmly to one another, and less in tissues where cells are less firmly attached to one another. In any region, however, where the tissue is uniform the pressure and separation of cells must be uniform, and no accumulation of tissue fluid in the form of lakelets would seem to be possible. Microscopic examination of embryos bears out this theoretical consideration; there are regions in which the intercellular fluid is large in amount, proportionately, and others where it is small. Where large, the mesenchyme or other cells are uniformly separated from one another, as for example in the umbilical cord, and the ventral body wall.

It is unjustifiable from our knowledge, or better, lack of knowledge, to speak positively about the stimuli which are responsible for the primary differentiation of organs or tissues, since with, perhaps, the exception of the formation of the lens as the result of the contact of the optic cup with the epidermis (7), there is hardly an instance in embryology of the satisfactory demonstration of the stimulus responsible for the primary differentiation of any organ or tissue (8). In this connection, results obtained in recent studies made on tissues, grown by the 'tissue culture' method of Harrison, are interesting. Harrison (9) found that primitive nerve cells send out processes into a medium consisting of coagulated lymph. The Lewises (10) obtained similar results with sympathetic nerve cells, in a medium of Locke's solution. Shipley (11) has found that undifferentiated heart muscle cells differentiate and start rhythmic contractions, in a medium of coagulated plasma. In all of these cases the cells were removed from their normal environment. Their continued development makes one sceptical of hypotheses as to the nature of formative stimuli, when such hypotheses are not supported by fact.

The only experimental evidence which has been proposed in support of the hypothesis that collections of lymph furnish

the stimulus to the formation of lymphatics, consists of the results of studies on 'experimental mesothelium,' by W. C. Clark (12). This investigator found that if solid blocks of celloidin are placed in the subcutaneous tissue of dogs, they became surrounded by flattened cells, which show, when treated with silver salts, black intercellular lines typical of flattened mesothelial or epithelial tissues. Solid globules of hard paraffin, injected into the cornea of rabbits, were surrounded by a layer of flattened cells. Flattened cells were also found to line 'dead spaces' in the tissue and artificial channels, such as may be induced by the ligation of the cystic duct, with formation of a mucous fistula.

Interesting as are these studies, in spite of the absence of evidence as to the source of origin of the cells which formed the flattened lining membrane, there appears to be no justification for the conclusion that we have thereby gained any information as to the mode of differentiation or growth of blood or lymphatic vessels. And yet, W. C. Clark concludes (page 316) that "Therefore the second hypothesis, premised in this article, is tenable, namely that the flat cells of serous surfaces and those lining blood vessels may regenerate from deep connective tissue cells, and do not necessarily arise from adjacent intact mesothelial or endothelial cells." Again McClure (13) refers to the results of W. C. Clark as bearing out "in a most decisive manner" the view that "the gradual increase in the amount of lymph received by the subocular sacs (in the trout) during the stage of their independence, results in the application of a constant and continuous pressure to the mesenchyme cells forming their walls, which in itself must be a positive factor in causing these cells to flatten out and gradually assume an endothelial form."

It is difficult to conceive how the results obtained by W. C. Clark can have any bearing on the problem which confronts McClure, unless the assumption is made that all mesothelial, endothelial, and epithelial membranes which line spaces or ducts have the same properties—an assumption for which facts furnish no justification. Surely blood-vessel endothelium has

properties which differ from synovial membranes, peritoneal membranes, or the lining membrane of the urethra, the bile ducts, or the gall-bladder.

There is, perhaps, a suggestion of support for the hypothesis in question in some of the results obtained in tissue cultures. Several observers—Harrison (14), M. R. and W. H. Lewis (15), Lambert (16), W. C. Clark (17) and others—have found that in bits of tissues, explanted to plasma or Locke's solution, membranes may be formed around solid bodies—such as along the cover-slip and around solid threads, as the threads of spider web, used by Harrison, and around droplets of fluid—such as may be formed, occasionally, in plasma preparations, by the retraction of the fibrous threads. The explanation of the formation of membranes around droplets of fluid is associated with the apparent inability of cells to grow into a purely fluid medium, without mechanical support, or as expressed by Harrison, their dependence on 'stereotropism.' The formation of such a membrane in tissue cultures is not to be interpreted as a reaction by flattening out, on the part of the culture cells, but rather, in all probability, as due to the fact that cells grow around the periphery of such a droplet. Moreover, it has not yet been possible to determine the origin of the cells which form membranes in tissue cultures. At present there appears to be no ground for claiming that the formation of membranes in this manner, furnishes us any information as to the mode of differentiation of blood-vessel or lymphatic endothelium.

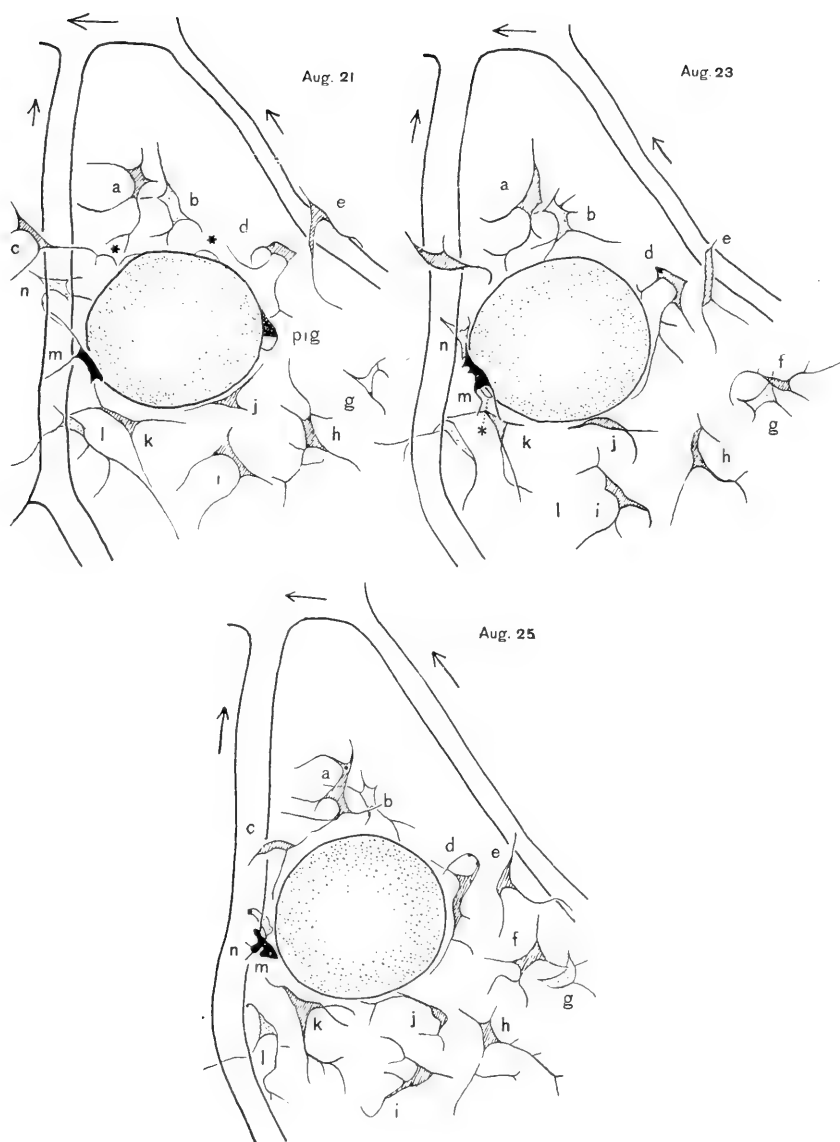
In order to put to the test the hypothesis that the differentiation of blood or lymph vessel endothelium may be stimulated by the mechanical pressure exerted on mesenchyme cells by accumulations of fluid, and to plan the test in such a way as to make it approach as nearly as possible the actual conditions supposed to exist, the present studies were started. The aim was to inject an inert fluid, in globules of size sufficient to press against the mesenchyme cells, into a region of embryonic tissue, in which the reaction of the cells could be watched in the living animal; to see whether a membrane were formed, and, if so, by what type of cell, and what would be the properties of such a

membrane, especially its reaction toward blood-vessel and lymphatic endothelium.

In order to simulate as nearly as possible the fluid whose presence is thought to excite the transformation of mesenchyme cells into lymphatics, and at the same time to have a fluid which would be inert, which would not be absorbed, which would merely exert a mechanical pressure, paraffin oil was selected for injection. The object chosen was the transparent fin expansion of the tail of young frog and toad tad-poles where it is possible to see the individual mesenchyme cells, as well as blood-vessels, lymphatics and leucocytes, and to watch their reactions in the living larvae, from day to day. The tad-poles were anaesthetized with chloretone (1:4000 to 1:5000) and small globules of oil injected into both fins, through fine glass cannulae, under the binocular microscope. The oil was sterilized by heating. In some cases the tadpole was washed in several changes of sterile water, but the results did not differ materially from those obtained when the only antiseptic precaution consisted in sterilizing the oil. The observations were made by a method previously described in detail (18)—the larva, anesthetized, was placed in a micro-aquarium, in chloretone of the proper strength, and the tube of the microscope tilted to the horizontal, to enable the tadpole to retain its normal upright position. The oil globules were of varying sizes, from 20 to 100 micra in diameter.

The larvae used were those of *Rana catesbiana* (bull-frog) and of Fowler's toad (*Bufo lentiginosus* Fowleri). The latter have beautifully clear tails, with very few pigment cells at the stages used, while the mesenchyme cells are far apart and stand out most clearly.

Since the oil could not be injected without a certain amount of injury, there were always some temporary effects of the injection, not attributable to the presence of the oil. These consisted principally of a more or less intense leucocytosis, probably caused by greater or milder degrees of infection. In some cases large numbers of leucocytes gathered about the globules, many of the leucocytes containing pigment. Several of the globules, around which the leucocytosis was most intense



were extruded. In case this did not occur, the leucocytosis gradually subsided until in most cases three or four days after the injection, the region surrounding the globule contained no more leucocytes than the other parts of the tail. In some instances, a slightly increased number of leucocytes near the globule continued for several days longer. From now on the globules remained apparently inert, so far as could be judged from the behavior of leucocytes in their vicinity. The longest time over which a globule was watched was 12 days.

In appearance the oil globules, when present in the tail, form spheres with the central portion clear and transparent and a dark periphery, with a sharp outline. Structures over the central portion can be seen most clearly. Thus it is possible, in case the diameter of the globule is sufficient to distend the skin slightly, to see the nuclei of the cells of the epidermis, and the details of other structures most distinctly. Many of the globules were oval in shape, immediately after injection. Later, after a day or two, they usually rounded up to a spherical shape though sometimes remaining slightly oval.

The behavior of the mesenchyme cells will now be described. In order to follow them with accuracy camera lucida records were made of all mesenchyme cells in the neighborhood of the globules, and their changes from day to day were noted. When a mesenchyme cell happened to be in the outer dark zone of the globule, it was difficult to make out its outlines. Occasionally only one or two of its processes could be clearly seen. The following of such cells, however, was made possible by the fact

Fig. 1 From larva of Fowler's toad, 9 mm. long. Four globules of paraffin oil injected into fin expansion of tail, on Aug. 19. Much leucocytosis about three of them, and all three extruded within forty-eight hours. There was very little leucocytosis about the fourth globule, of which three drawings are shown, two, four, and six days after injection. This globule was in the ventral fin. The mesenchyme cells in the immediate vicinity of the globule are shown. The letters, *a*, *b*, etc., indicate the same cells. * leucocytes against the globule; *pig.L.*, pigmented leucocyte against the globule. The mesenchyme cells were in three different planes; those nearest the observer are represented in solid black, those furthest away are dotted, while those in the midst are cross-hatched. Enlarged 267 times. Drawn with camera lucida.

that the globules from time to time shifted their position slightly, so that a cell, at one time not clearly seen, later could be clearly outlined. This applied to only a very few cells, particularly in the toad larvae, because of the relative rarity of their mesenchyme cells. In view of the descriptions in the literature of the reaction of connective tissue cells to the pressure exerted by foreign substances, the behavior of the mesenchyme cells was a great surprise. It was expected that the cells near the globule would flatten out on its surface and form a membrane. On the contrary, the mesenchyme cells apparently paid no particular attention to the globules. They maintained their identity as 'star-shaped' cells, with thickened central portion and branched processes, and their property of slow progression, described in an earlier paper. That the mesenchyme cells are not influenced by the pressure exerted by the globules was brought out quite strikingly in one instance in which the globule shifted its position in such a way as to come to lie against a mesenchyme cell which had been at a slight distance from the globule. For a day or two it was rather difficult to make out the outlines of the cell. The globule then shifted its position in the opposite direction, and the mesenchyme cell could now be seen clearly, apparently unchanged, at a slight distance from the globule. Occasionally there are to be seen, over the clear part of the globule, if the globule is of sufficient size to distend the skin slightly, one or two flattened cells which, at first glance, might be interpreted as cells flattened out by the pressure of the globule. Such cells were seen over only a few of the globules, and their explanation was obvious on studying other parts of the tail, at a distance from the globules. Such flattened out mesenchyme cells appear more or less evenly distributed, lying just below the epidermis, and are not particularly associated with the oil globules. That they are associated with the skin and not with the oil globules, is shown by the fact that none are present over the majority of the globules, and also by the fact that, if the globules shift, these cells maintain the same position with reference to the skin, while they are left behind by the oil globules (fig. 4). There are no other appearances on

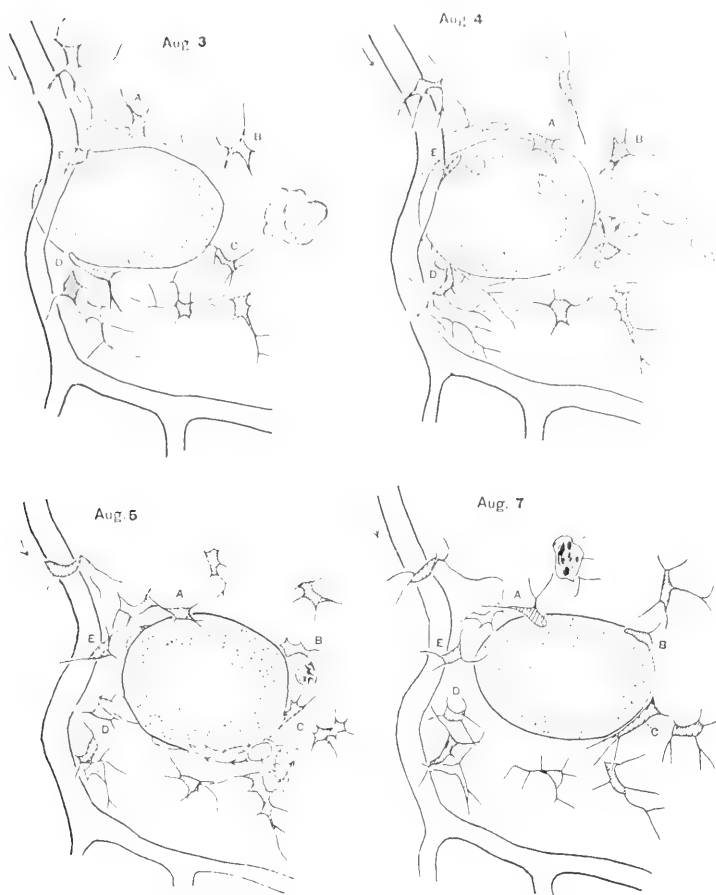


Fig. 2 From larva of *Rana catesbiana*, 10.5 mm. long. Two globules of paraffin oil injected into the ventral, and a small globule into the dorsal fin; all three remained. Much leucocytosis around each. The first drawing (Aug. 3) was made immediately after the injection. Small mass of cellular debris is shown, in the path of the injection. Mesenchyme nearest the globule lettered as in fig. 1. In drawings Aug. 4, 5, and 7, some of the leucocytes about the globule are shown. In drawing of Aug. 5 are shown the successive positions taken by a leucocyte as it moved to the globule, moved along the surface of the globule a short distance, and then moved away. The shifting of the globule is clearly seen, by comparing its relation to the blood vessel. The cells *d* and *e*, which lie close to the globule Aug. 3 and 4, are left behind, while the cells *b* and *c* are approached by the shifting of the globule.

Enlarged 267 times. Drawn with camera lucida.

the part of the mesenchyme cells which even remotely suggested a flattening out. The fact that some of the globules watched shifted their position would also indicate that no surrounding membrane had been formed, for a membrane would prevent such movement.

The behavior of wandering cells towards the globules was watched with interest. As already stated there was a leucocytosis of greater or less intensity following the introduction of the globules, for the first two or three days. Leucocytes, most of them small and clear, others larger, and containing pigment, collected around the globules. Many of them flattened themselves out on the surface of the globule, or formed irregular humps on the profile. Occasionally such a flattened leucocyte formed a thin, circular structure, with nucleus visible over the clearest, central portion of the globule. Such a cell, coupled with the irregular humps on the profile, if seen only at one stage, and not followed, might well give the impression of membrane formation by wandering cells. When, however, such cells were followed, it was seen that they gradually moved away (fig. 2). The humps on the profile changed shape, with each drawing, even when the records were made several times daily, while the flattened cells on the clearer part moved away. After four or five days most of the globules were quite free from the presence of leucocytes. In order to be sure of the behavior of the wandering cells, some were watched intensively. They were seen to move, with the typical amoeboid type of progression, up to the oil globule, to flatten themselves out on its surface and again move away. The impression was gained, that, had sections been made at a time when the leucocytes were flattened on the globule, they might have been interpreted as forming a membrane, an interpretation which the study of the living shows would be quite unjustifiable.

Chromatophores which are present in large numbers in the dorsal fins of bull-frog larvae and to a somewhat less extent in the ventral fins of the same larvae, sometimes wrapped around the globules with their long branched processes, when the glob-

ules were injected near them, but did not form a definite membrane.

Since the mesenchyme cells failed to form a membrane around the globules, the second part of the inquiry, namely, the reaction of such a membrane, if formed, toward the lymphatics or blood-vessels in their vicinity, could not be followed. It was of interest, however, to observe the reaction of blood-vessel and lymphatic endothelium to the oil globules. In one case a particularly favorable opportunity was afforded to study the reaction of the blood-capillary, since the globule pressed against a blood-capillary, forcing it to make a bend in its course (fig. 4).

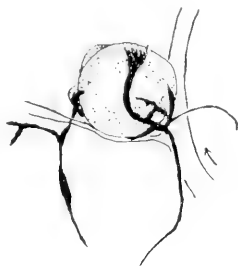


Fig. 3 From same *Rana catesbiana* larva from which figure 2 was taken. To show relation of pigment cells to globule. This small oil globule was injected into the dorsal fin, on Aug. 3; the drawing was made Aug. 5.

Enlarged 267 times. Camera lucida.

The capillary then appeared to wrap around a part of the globule. In this position the capillary showed no tendency to give off cells which might grow around the globule, but instead remained as a distinct vessel. The circulation of blood cells through it, which was at first interrupted, was later resumed. Blood-capillaries and lymphatics near the oil globules showed no tendency to grow toward it, or to send out sprouts to it.

The results of this study, then, indicate that, aside from the temporary inflammatory reaction, due probably to the injury and to the bacteria introduced at the time of injection, the presence in the fin of the tad-pole's tail of injected globules of paraffin oil, of sufficient size to cause a distension of the tissues, fails

to stimulate the formation of membranes about the globules, on the part of mesenchyme cells, wandering cells, or of blood-vessel or lymphatic endothelium.

These results are in disagreement with those of W. C. Clark (19), already mentioned. They are, however, in agreement with the older findings of E. Juckuff (20) who found that soft paraffin, injected subcutaneously, travelled long distances from the injection site, showing that no membrane was formed about

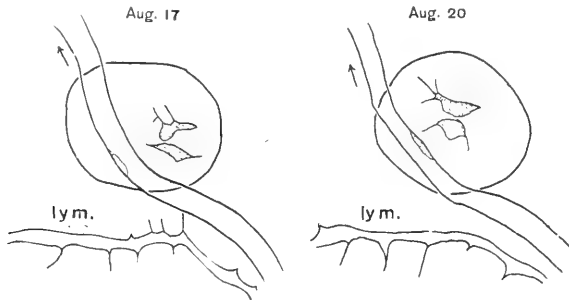


Fig. 4 Globule of paraffin oil injected into ventral fin of tail of *Rana catesbiana* larva on Aug. 13. The globule rested against a blood-capillary, forcing it to bend slightly. The two sketches shown, made four and seven days respectively after the injection, show the relation of the globule to the blood capillary, to a nearby lymphatic, (*lym*) and to two mesenchyme cells which happened to be under the epidermis immediately over the globule. Note that the globule has shifted to the right, so that the two mesenchyme cells, which on Aug. 17 are over the right part of the globule, are over the left central portion Aug. 20. Other mesenchyme cells not shown.

Enlarged 267 times. Drawn with camera lucida.

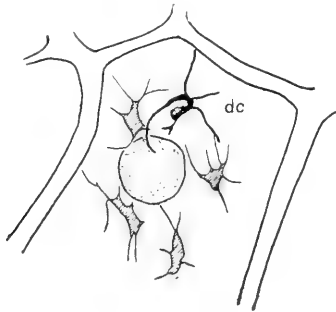


Fig. 5 Same larva as figure 4. Small globule injected in dorsal fin on Aug. 13. Drawing made nine days later—on Aug. 22, *d.e.*, pigment cell. Enlarged 267 times. Drawn with camera lucida.

it—a finding verified by MacCallum (21). It will be remembered that the attempt was made, in selecting the substance to be injected, to find something which would simulate the supposed lakelets of tissue fluid whose presence are held by Huntington and McClure to stimulate, merely by the mechanical pressure which they are supposed to exert, the mesenchyme cells to form membranes. It is obvious that in point of size and consistency the small globules of oil much more nearly reproduce the supposed conditions than the relatively enormous pus pockets, or the relatively huge solid blocks of celloidin or the globules of hard paraffin. It is also obvious that in a transparent object, like the tad-pole's tail, where the individual cells may be seen with great clearness and the reaction process watched in the living animal, the conditions for observing what happens are much more favorable than in the case of the other experiments referred to.

Since, then, the action of pressure alone fails to stimulate the formation of membranes on the part of mesenchyme cells, an important link in the argument used in favor of the origin of lymphatics from mesenchyme cells, as presented by Huntington and McClure, drops out.

A certain feeling of disappointment must be confessed, that the mesenchyme cells failed to respond to the presence of the oil globules by the formation around them of membranes, as it was expected they would, because of the desire to see what would be the reaction of such membranes toward blood-vessels and lymphatics.

It is true that cells derived from the middle layer or mesoderm differentiate at various stages into pavement epithelium, or endothelium, other than that which lines the blood and lymph vascular systems. Among such may be mentioned the lining of the large cavities pleural, peritoneal, and pericardial, the lining of bursae and synovial membranes, and the outer layers of tendons and fasciae. It should also be remembered that the same middle embryonic layer differentiates into smooth and striated muscle, into cartilage and bone, into blood cells, and other types of tissues. Each of these tissues has certain

modes of reaction, a specific life history, the property of responding each in its own individual way, to various stimuli. To transfer the modes of reaction of one set of tissues derived from the mesoderm to another set is quite unjustifiable. To be more specific, it is not justifiable to claim that, if connective tissues, in adult animals, are capable of forming membranes about solid foreign bodies, or large accumulations of fluid, or if membranes form around liquid vesicles in the midst of coagulated lymph in tissue culture preparations, then lymphatics arise as the result of the pressure exerted by accumulated lakelets of lymph. It is even unjustifiable to transfer to lymphatics the properties of structures which resemble them morphologically so nearly as blood-vessels, for, while there are many points of similarity between the modes of reaction of the two, there are also striking differences.

It is conceivable that future studies may reveal the various stimuli which are responsible for the primary differentiation of tissues and organs. For the lymphatic endothelium it would seem a more hopeful field to investigate the chemical nature of the intercellular fluid, to see whether any evidence can be gained as to the collection there of especial chemical substances which stimulate its differentiation. To propose such an hypothesis at the present time, however, would be pure speculation.

Much confusion has arisen because quite different structures have been grouped together under the name of endothelium or mesothelium. It would seem that the time is ripe to separate these different forms of flattened lining cells under different names. If, for example, we could speak of blood-vessel endothelium as *Haem-angiothelium*, and of lymphatic endothelium as *Lymph-angiothelium*, or some equally specific names, and if distinctive names could be selected for the other forms of pavement epithelium, much of the confusion would disappear.

In conclusion, it is a pleasure to express my gratitude to the Marine Biological Laboratory at Wood's Hole, where these studies were made, for generously granted laboratory facilities.

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AN ANOMALOUS URINOGENITAL SYSTEM IN A DOG

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TWO FIGURES

Anomalies of the urinogenital system are frequent and have ceased to attract much attention. Few, however, present features of such embryological interest as the following case. Because of this and on account of its value, as illustrating the physiological adaptability of one system to the needs of another, this case seems worthy of mention. My acknowledgment is due Dr. L. B. Arey for valuable suggestions regarding the embryological considerations.

On opening the abdomen of a dog it is commonly observed that the bladder is large and lies almost entirely in the abdominal cavity. In a medium sized mature female, selected at random for the purpose of obtaining certain tissues used in a research problem, the bladder lay deep in the pelvic cavity and was rather small, the size and shape being that of a walnut. On palpation it felt extremely firm, much as though it were a solid mass of tissue. A longitudinal incision through its wall revealed but a very small lumen, less than 1 cm. in diameter and 2 cm. in length.

Two broad ligaments passed from this bladder over the rectum and gained attachment to the front of the sacrum. One ligament was considerably longer than the other, due to the bladder lying ventral and to the left of the uterus instead of directly ventral as is normally the case. Except for these two ligaments and a slightly shortened urethra which merged into the left wall of the urinogenital sinus, other connections with the bladder could not be established.

¹Contribution No. 40, May 15, 1916.

These findings led to an examination of the kidneys, which were found to be normal in shape, size and position. Each possessed one short ureter, the right being 6.5 cm. and the left 7.2 cm. in length.

Originating in the pelvis of the kidneys the ureters coursed downward over the psoas muscles and passed one on each side into the horns of the bicornuate uterus. This union occurred about a centimeter below the place where the short Fallopian tubes merge into the uterine horns. The uterine horns and the uterus were not soft and pliable as is usual but were hard and rigid and on making a longitudinal incision through their walls, were found to be filled with *débris*, composed mainly of desquamated epithelial cells.

The structures in this region were surrounded and some deeply imbedded in a mass of fibrous and adipose tissue. This appeared to form a common capsule which covered the union of the ureters with the uterine horns and extended over the Fallopian tubes including the ovaries, becoming at this connection part of the ovarian bursa.

The ovarian bursa exists normally in the dog as a separate fold of peritoneum covering each ovary. This is usually covered by adipose tissue but opens through a small slit-like opening into the abdominal cavity.

It has been mentioned that the urethra was slightly shorter than normal and passed from the left into the wall of the urinogenital sinus. This relation of the urethra to the urinogenital sinus and the original location of the bladder explains why in the accompanying figure (fig. 1) the bladder is shown as lying between the uterus and rectum, instead of ventral to the uterus as is normal.

Histological preparations of the bladder show a slightly changed epithelial lining, consisting in two to three layers of low cuboidal epithelium. The uterine surface epithelium instead of being high columnar in type is pseudo-stratified. The deeper glandular epithelium, however, is the same as in a normal dog's uterus. The ovaries which were deeply imbedded in their ovarian bursae on sectioning showed nothing atypical, with the exception of

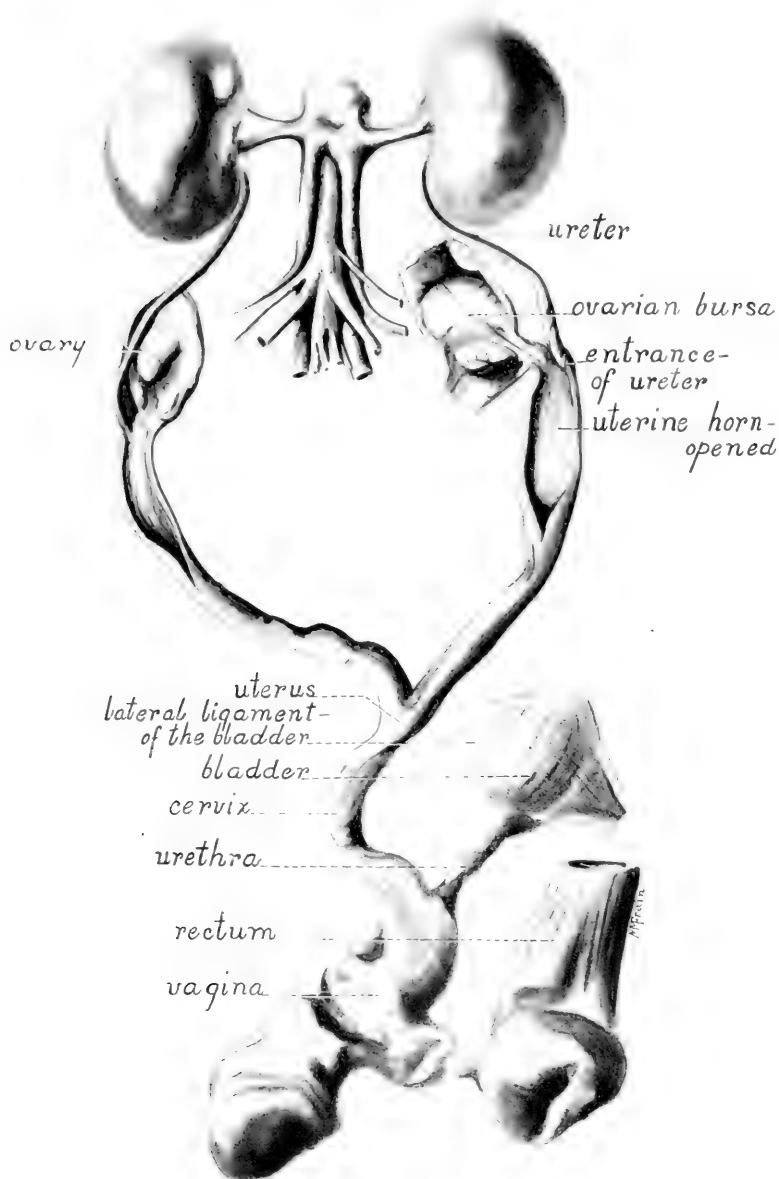


Fig. 1 Ventral view. Ureters shown as they enter both horns of uterus. Ovarian bursae and upper end of uterine horns opened. Bladder small and abnormal in location.

a slightly more fibrous stroma than is usual. Several large Graffian follicles present indicated a normal functional activity of the ovaries. Histologically, then, practically nothing unusual exists, the anomaly being one of gross anatomy, this consisting in a union of the urinary with the reproductive tract, the fusion of ureters and uterine horns leaving the bladder as a cul de sac which leads through the urethra into the urinogenital sinus.

EMBRYOLOGICAL CONSIDERATIONS

The structures involved here are embryological derivatives of the mesonephric ducts, metanephros, Muellerian ducts and cloaca. That the cloaca developed normally is indicated by the presence of a rectum, bladder, urethra and urinogenital sinus. The presence of the uterus, tubes and vagina likewise indicate the normal development of the Muellerian ducts. The anomaly then must be due to a defective embryological growth of the mesonephric ducts and their derivatives, with a deficiency in the development of the nephrogenic cord as a possible causal stimulus.

In pig embryos of approximately 5 mm. length the mesonephric ducts give rise to the ureteric anlage of the metanephros where the ducts bend to join the cloaca. But that the ureteric anlages do not always originate at this bend is indicated by the frequency of double or triple ureters. In these instances the first ureteric bend develops usually into the ureter most normal, while the rest show evidence of slowed or mal-development. From these cases it may be assumed that the ureteric anlage need not necessarily arise at a definite location but can occur at any point along the ducts.

Figure 2, a very diagrammatic sketch, shows both the Muellerian and Wolffian ducts leading into the urinogenital sinus. The approximate position where normally a single metanephric anlage arises from the Wolffian duct is indicated by (A). However, in man as many as six such anlages have been observed. While in these instances the anlage corresponding to (A) develops into the adult ureter the possibility exists that a more

cranial anlage, for instance (*B*), may become the functional ureter.

Reference to figure 2 will show the lower part of the Wolffian duct not cross-hatched. This portion which extends from the normal ureteric anlage on downwards is during further development drawn into the urinogenital sinus. Through this fusion the ureters receive their normal connection with the definitive bladder which develops partly out of this portion of the sinus.

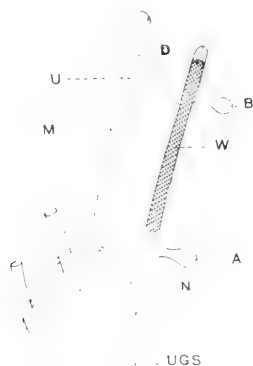


Fig. 2 Diagrammatic sketch. *M*, Muellerian duct; *W*, Wolffian duct; *A*, location of normal metanephric anlage; *B*, possible upwardly displaced metanephric anlage; *D*, extended ureter merging into the Muellerian duct at *U*; *N*, portion of Wolffian duct taken into wall of urogenital sinus; *UGS*, cross-hatched portion of Wolffian duct degenerates.

If in this particular case, however, the ureteric anlage did not develop low enough to be included in that lower portion of the mesonephric duct then just as soon as the normal degeneration of the upper part of the Wolffian duct occurred, the upwardly displaced anlage (*B*) which developed into the ureter was without connection with the urinogenital sinus. Being thus isolated it seems probable that the ureter (*D*) extended to the nearby Muellerian duct and merged into it at (*U*). This established an outlet into the urinogenital sinus.

The above is offered as one possibility to which the present anomaly may be due. It is entirely hypothetical as any con-

sideration of this case must be. Because of this a further explanation may possibly be found in the following. In addition to those known embryological facts mentioned above it should be recalled that in embryos of 8 to 11 mm. length the Muellerian ducts develop caudalward beneath the epithelium of the mesonephric fold. Reference to dissections of the pig embryo show the Muellerian ducts lying very close to the Wolfian ducts so that the occurrence of a more or less complete longitudinal fusion of these two ducts seems not impossible.

The establishment of this anomalous union of ureters and uterine horns presumably occurred early in the development of the animal. Since in the female, during the normal development the mesonephric ducts degenerate and disappear almost entirely, it may be assumed that in this case these ducts gradually fused with the Muellerian ducts. It may be that in this way an early connection occurred on either side between the Muellerian duct and an upwardly displaced ureter.

The question may be raised why a metanephros thus displaced should have abandoned the mesonephric duct and appropriated a new outlet by way of the Muellerian ducts? It may be that the functional need of maintaining the patency of the mesonephric duct was ineffectual compared with the tendency toward atrophy and consequent occlusion which the cranial portion normally shows. This query becomes all the more pertinent in view of the recent report by Bremer (*Jour. Anat.*, vol. 19, '16) that in the cat the mesonephros maintains its activity until the permanent kidney assumes the excretory function. This being true of the cat it is more than probable that it also exists in the dog since both belong to the Carnivora.

SENSORY ELEMENTS IN THE HUMAN CEREBRAL HYPOPHYSIS

W. SOHIER BRYANT

ONE FIGURE

In the past few years, the greater part of the work relating to the cerebral hypophysis has been of a therapeutic, a clinical or a surgically experimental nature, and the interest aroused in these aspects of the pituitary has tended to obscure the fact that certain histological elements in the structure of the organ still remain a mystery. The following report re-introduces the subject of the sensory elements of the hypophysial cavity, of which I have made a careful examination in human specimens: These sensory elements occur in maculae, which, in sagittal sections of the pituitary are seen situated on the posterior wall of the cavity, and sometimes, apparently, on the anterior wall. The maculae are composed of tall columnar ciliated sensory cells interspersed with bipolar cells, which have their nuclei towards the periphery; whereas in the ciliated cells, the nuclei are near the base which terminates in a caudal prolongation. Between these caudal processes of the ciliated cells, there is a layer of round cells, resting on a thin basement membrane. An area of ciliated cuboidal cells occurs at the margins of the maculae. I have found these sensory cells in all the freshly hardened human hypophyses that I have examined, except those in which the parenchyma had been almost completely replaced by connective-tissue; the sensory cells are moreover encountered even in pituitaries which have undergone very extensive pathological change. In their gross arrangement, the sensory elements of the hypophyseal cavity are suggestive of the sensory elements of the maculae acousticae.

Gentès (3), in his examination of the hypophysis of cats and dogs, found in the juxta-nervous layer, a stratified cylinder

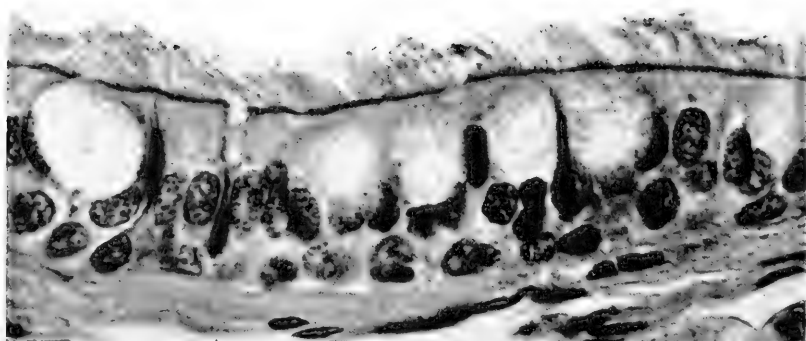


Fig. 1 Sensory epithelium of the hypophyseal cavity of a human adult. Stained with hematoxylin-eosin.

This material was procured through the kindness of Dr. William Mabon and the assistance of Dr. Clarence O. Cheney, Pathologist of the Manhattan State Hospital; the work was done in the New York Psychiatric Institute. Special thanks are due Dr. Charles Bates Dunlap for his technical assistance and supervision of the work.

epithelium resembling certain sensory epithelia. Cajal (1) has noted numerous special bi-polar cells in the epithelium adjacent to the nervous lobe in the hypophysis of the rat. Pirone (4), on the basis of his examinations in the cat and the dog, confirms the findings of Gentès: the cylindrical epithelium, in its superficial layers, presents the structure of the supporting cells of sensory epithelia. Gemelli (2) finds that the posterior wall of the hypophysial cavity, in the guinea-pig, is composed of a layer of cylindrical cells, and other cells which he calls supporting cells. The only reference in the literature to sensory elements in the human hypophysis is given by Tello (5), who mentioned that he found sensory epithelium in his human specimens, but omits to give any adequate description of it.

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A METHOD FOR MICRO-INJECTION

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ONE FIGURE

After spending several months in an effort to secure satisfactory results and economy of material with methods used by other workers, the writer devised the following apparatus which is very easily arranged and does the work without waste except for accidents due to inexperience or carelessness in handling.

Direct pressure is secured from a good (50c.) De Vilbiss atomizer bulb. The bulb lies on the floor and is kept from rolling by a crocheted net cover. The pressure is transmitted by a red rubber tube $\frac{3}{16}$ of an inch in diameter. After the transmitter reaches the table a short glass tube (h) is inserted in it and a cord (g) run from this to the upright of a small stand with a heavy base to keep these parts in position. Eleven inches further on a glass T (r) is inserted, to the stem of which a thistle tube is joined by a short piece of rubber tubing (p). Eighteen inches more of the transmitter end with a short glass injection tube (c) which has the outer end drawn into a capillary tip at right angles to itself. A sliding rod (i) passed through the pivoted top (b) of the upright of the stand makes a convenient support for the end of the transmitter. A piece of cork (a) on the pointed bent tip of the rod gives enough friction to keep the tube from slipping and still allows it to be readily moved in either direction or removed for use, with one hand. A low Stender dish (d) for the injection fluid and a tumbler of water (b) for cleansing and protecting the injection tube are placed near the stand. A small bull-dog clip completes the essential outfit. This clip placed below the T tube allows the injection tube (c) to be more quickly filled through the capillary tip by applying suction with the mouth through the thistle tube. It is then placed at (p) between the T and the thistle tube when pressure for injection is to be applied.

It will be noticed that this apparatus allows of modifications to suit the worker or to adapt it to special conditions. It does not seem necessary to describe dissecting stands or microscopes but a few words as to the handling of the apparatus and the preparation of certain objects may save time, temper and material for persons attempting injection for the first time.

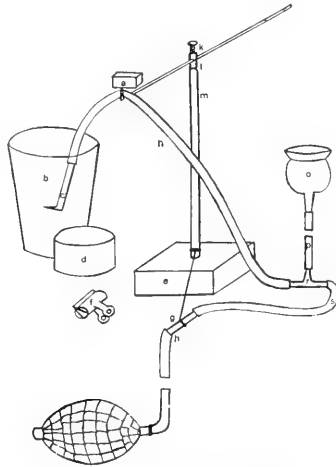
In regard to the apparatus:

1. It is desirable to have a number of injection tubes ready for use. The writer has found it easiest to make these over a fish-tail burner, or a minim burner. Sufficient thin tubing for two tubes is heated at the centre for the shortest possible distance and when sufficiently soft, bent and quickly drawn apart. When one has secured a good tip, it is well to heat the other end enough to smooth it so that it will not cut the rubber tubing on insertion.

2. Remember that capillary tubes are very easily broken. When not in use the injection tube should hang freely in the tumbler where it will be safe from accidents.

3. The tip of the injection tube is very easily clogged. An injection fluid from which there may be any deposit should not be left in the tube a long time. Higgin's waterproof ink will also stand strong dilution with ammoniated water, four drops to the ounce according to their directions. If clogging occurs with the ink, egg albumen or shreds of tissue, to let the tip soak in a strong solution of NaOH often makes it possible to clear it and the NaOH may be easily washed out with water.

4. The clip should be removed when the apparatus is not in use.



To prepare small chick or similar embryos:

Keep the egg in the position in which it is found at the time of use. Remove as much of the albumen as possible by making a small opening on the lower surface and another on the top. The lower opening must be closed as soon as the yolk membrane appears. A piece of paper held in place by one finger will answer. The upper half of the shell may then be removed and the embryo exposed. Irrigation with a warm normal salt solution helps to remove the albumen and so to prevent the clogging of the capillary tube. It is also very desirable to remove the vitelline membrane over the embryonic area if possible. The shell containing the embryo thus prepared, can easily be steadied in a low glass dish with a low raised ring near the centre. Such may sometimes be found at the ten cent stores. A brass curtain ring in a smooth dish would answer the same purpose.

Although the apparatus and method are so simple, it is desirable for any one who has never done this kind of work to practise with inexpensive material in order to gain some facility, before trying to handle that which is rare or costly.

THE PROLONGED GESTATION PERIOD IN SUCKLING MICE

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INTRODUCTION

A short paper by Prof. J. F. Daniel ('10) on white mice, and a later paper by Dr. H. D. King ('13) on white rats, have shown that in these two animals the gestation period is lengthened when the females suckle young during pregnancy. Daniel found a fairly definite relation existing in mice between the degree of prolongation of the gestation period and the number of young being suckled, about one day being added to the period of pregnancy for each of the nursing young. King, on the contrary, found that in rats the gestation period is normal "if the female is suckling five or less young and is carrying five or less young," while "the gestation period may be prolonged from one to six days if an albino female, suckling five or less young, is carrying six or more young," and "the period of gestation is always prolonged when a female is suckling six or more young."

Professor Daniel offered no explanation of the prolongation of the gestation period in suckling white mice other than stating the possibility that it might be due to lactation, either delaying ovulation or generally slowing down embryonic development, with the latter as the more probable. Dr. King, however, states positively that ovulation in albino rats is not delayed by lactation, but that the suckling of a litter that contains six or more young seems to lessen the food supply to the foetal young and so retards their development.

The present work has been in progress for nearly five years, and certain phases of it are still being investigated. White

mice only have been used, and the problem of prolonged gestation in suckling females has been approached from the embryological standpoint in the attempt to determine the detailed facts involved.

It was evident at the start that exact criteria for determining the stage of development of any embryo must be established, and, while Sobotta ('95) gives the time relations of development from ovulation to implantation, the series of eggs and embryos of known age prepared especially for this work includes sets from both suckling and non-suckling females, spaced at about twenty-four hour intervals from the time of parturition to the birth of the following litter. In each case the age of the specimens was definitely established by placing in individual cages all pregnant females, together with a male, looking every day for new litters, removing the male in every instance the day following the birth of the litter, and, if the female was to be used for the non-suckling series, removing all the young as soon as found. The age of eggs and embryos found in these females was then reckoned from the birth of the preceding litter, e.g., the day the litter was found is the first day after parturition.

THE DEVELOPMENTAL CYCLE IN NON-SUCKLING FEMALES

The development, in non-suckling white mice, of eggs and embryos is usually as indicated below, the exception being the very rare instances where the males probably paired with the females much earlier than the average time, following parturition, and in consequence the developing eggs or embryos are slightly ahead of this schedule. Cases of this kind number only three in eighty sets of eggs and embryos studied. It should be noted, further, that as a rule (one exception in fifty instances) when the males were left with the females the state of development of eggs and embryos was the same as in cases where the males were removed twenty-four hours after parturition. The single exception found was a set of normal eggs in the two-cell stage found in a female six days after parturition, a male having been in the cage with her continuously since a time preceding the birth of the litter.

Parturition. White mice give birth to litters at all hours of the day and night, but much more commonly at night or, according to Long and Mark ('11), in the early morning.

1 day post-partum. If the female white mouse comes in heat, as is the rule during the warmer part of the year (April to October) and the new born young are not suckled, within twenty-four hours after parturition ovulation and pairing (provided a male is present) will occur.

2 days post-partum. The eggs have been fertilized in the upper third of the Fallopian tubes and the first cleavage has occurred.

3 days post-partum. The eggs are still in the two-cell stage.

4 days post-partum. Cleavage is again in progress and morulas of 8 to 10 blastomeres are found at this time.

5 days post-partum. Morulas of 12 to 16 blastomeres. At the close of this day the morulas develop a central cavity, thus becoming blastulas, and at the same time they pass from the Fallopian tubes into the horns of the uterus.

6 days post-partum. Blastodermic vesicles lie free in the horns of the uterus.

7 days post-partum. The blastodermic vesicles are now implanted in proliferated masses of uterine cells which completely obstruct the lumen. The embryos themselves are in the 'egg-cylinder' stage, the 'cylinder' almost filling the vesicle and possessing a single, undivided cavity.

8 days post-partum. The egg-cylinder in embryos of this age has its lumen divided into three cavities.

9 days post-partum. The embryo now possesses a medullary groove, which is open except at the extreme anterior end.

10 days post-partum. Embryos of this age have the medullary groove closed in its anterior half, and for the first time show a heart.

11 days post-partum. The medullary groove is now closed for more than half its length; the optic vesicles are budding off from the brain; and the auditory vesicles appear as cup-shaped depressions in the ectoderm.

12 days post-partum. The medullary groove has closed except at the extreme posterior end; the auditory vesicles are almost or

entirely closed. The fore limb buds are present, together with the first nephric tubules and the anlage of the liver.

13 days post-partum. At this age the embryo is a decidedly complex organism, and from this time on the daily changes are rather matters of detail than the appearance of entirely new structures. The characteristic features of this particular stage of development are these: a well developed cranial flexure; optic vesicles completely separated from the brain, invaginated, and showing the beginnings of lens formation. The nasal capsules are visible; the liver has developed into a distinct organ; hind limb buds are present. The embryo has the anlagen of the lungs, and a few pancreatic tubules; the auditory vesicles have withdrawn from the surface and are connected by nerve fibers with the brain. Along the free border of the kidneys, especially at the posterior end appears the genital ridge with a few large cells with large round nuclei, the primordial germ cells, scattered through a much larger number of smaller, epithelial cells.

14 days post-partum. The eyes have developed to the stage where the lenses have a solid, clear core. Dense masses of connective tissue foreshadow the future location of the bones of the limbs, girdles, and ribs. Whisker follicles are present; also the semi-circular canals of the ears. The kidneys possess definite boundaries. The nuclei of the red blood corpuscles are smaller and stain less deeply than in earlier stages, while their cytoplasm shows a faint indication of haemoglobin. The first indications of teeth follicles are found in embryos of this age; also the anlagen of the thymus and thyroid glands. The gonads differ from the preceding stage merely in having more of the primordial germ cells in the genital ridges.

15 days post-partum. Embryonic cartilage cells constitute the most striking characteristic of this stage, clearly differentiating embryos of this age from all younger specimens. Other features are the fewer and smaller blood spaces in the liver, as compared with fourteen-day embryos; the deeper straw yellow color in the cytoplasm of the red blood corpuscles, together with a few which are non-nucleated; the well-developed anlagen of

the teeth; and the presence of cartilage cells in the floor of the cranium. Sexual differentiation is present in embryos fifteen days post-partum, male specimens showing gonads in which follicle formation has already started, while the female gonads preserve the earlier condition of primordial germ cells scattered through a mass of epithelial cells.

16 days post-partum. Differential characteristics now become still more matters of detail and of direct comparison with earlier stages, however, embryos of this age differ from all younger ones in having a decidedly transparent cornea. The heart has assumed its final shape. The pancreas is a clearly defined organ. The nasal capsules open into the front part of the mouth, while the naso-pharynx is connected with both the nasal capsules and the back part of the mouth. The gonads show no marked change from those of fifteen days embryos.

17 days post-partum. The chief characteristic of embryos of this age is the commencement of ossification around the rib cartilages. Nucleated red blood corpuscles are very scarce. The tongue possesses conspicuous striated muscle cells, stratified epithelium, and at least one circumvallate papilla. The nasal capsules have lost their direct connection with the mouth, but the nasopharynx opens into both the anterior and posterior regions of the mouth. The anlagen of the cartilaginous rings of the trachea are present. The testes have tubules with a peripheral layer of small cells while the larger primordial germ cells occupy the lumen. In the ovaries an ingrowth of connective tissue is noticeable.

18 days post-partum. Ossification of the cartilaginous skeleton is now the striking feature, and the membrane bones of the upper jaw, hard palate and roof of the skull are also being formed. The testes show a considerable amount of connective tissue between the tubules, while the ovaries differ from those of the preceding stage only in that they project further into the abdominal cavity.

19 days post-partum. The ribs of embryos of this age have an outer shell of bone, and the underlying cartilage is being torn down to make a marrow cavity. There is a marked spongy

structure in the lungs; the eye balls show a differential curvature in the cornea and sclerotic; the naso-pharynx no longer opens into the front of the mouth. The testes show no change, but the ovaries are more spherical, and the ingrowth of connective tissue has forced most of the germ cells toward the periphery.

20 days post-partum. The iris and choroid of the eyes first show pigmentation at this time, and lymphoid tissue appears in the tonsils. One litter of four animals was born twenty days after the birth of a preceding litter and grew to maturity.

21 days post-partum. The feature of this stage of development is the ossification of the metacarpals and metatarsals. The testes have a well organized tunica albuginea and show less space between the individual tubules than in twenty-day specimens, while in the ovaries the primordial germ cells, or oogonia, are of varying sizes, the largest of them beginning to form follicles about themselves.

22 days post-partum. The embryos in non-suckling white mice have now completed their intra-uterine development, and parturition occurs.

Such, in brief, is the record of development from day to day of white mouse embryos carried by females not suckling young. More extended study and more material would certainly yield many more details, but the above data are sufficient basis for estimating the age of embryos of unknown history, provided only they come from non-suckling females. Also the evidence collected is adequate for comparison with the facts noted below concerning the development of embryos carried by suckling mothers.

THE DEVELOPMENTAL CYCLE IN SUCKLING FEMALES

White mice are able to become pregnant while lactating but when suckling a litter only about one female in five undergoes a complete pregnancy; those which do not complete a pregnancy either failing to ovulate (the majority of cases) or the fertilized eggs developing normally until shortly after implantation in the uterus, when they die and are absorbed (the minority of

cases). In some instances we find another state of affairs, certain ones of a set of implanted embryos undergoing normal development while others die and are absorbed, a very rare condition in non-suckling females.

Suckling females which are not going to skip an ovulation cycle shed their eggs within twenty-four hours of parturition as do non-suckling females. These eggs are then fertilized and divide according to the same time scheme as given above for eggs in non-suckling females, being in the two-cell stage on the second and third days following parturition, morulas on the fourth and fifth days, and blastulas lying free in the uterus on the sixth day post-partum.

Now comes the point of greatest interest in this investigation. The fertilized eggs in non-suckling females, as stated earlier in this paper, become implanted in the uterus at the close of the sixth day post-partum, the fertilized eggs in suckling white mice, on the contrary, lie free in the lumen of the uterus from the sixth to the end of the fourteenth day following parturition. The material on which this statement is based comprises serial sections of the entire uterus of ten females, suckling from three to eight young, killed at various times from the sixth to the fourteenth day post-partum, all of which show normal blastulas lying free in the uterus, with no sign of any reaction in the adjacent cells of the uterine epithelium (table 1).

TABLE 1

Data regarding all suckling white mice from which embryos were obtained from the sixth to the fourteenth day following parturition

AGE IN DAYS POST-PARTUM	STAGE OF DEVELOPMENT	NUMBER OF SUCKLING YOUNG
6	Free blastulas in uterus	6
7	Free blastulas in uterus	4
8	Free blastulas in uterus	5
9	Free blastulas in uterus	3
10	Free blastulas in uterus	5
10	Free blastulas in uterus	8
11	Free blastulas in uterus	6
12	Free blastulas in uterus	4
13	Free blastulas in uterus	4
14	Free blastulas in uterus	5

Here evidently is one, and perhaps the main cause of the prolonged gestation in suckling white mice. Ovulation, fertilization and early cleavage stages occur at the same time intervals in both suckling and non-suckling animals, but in the suckling females the uterine cells will not react to the eggs and enable the latter to become implanted while the mammary glands are withdrawing the surplus nourishment from the parent organism. In support of this statement it should be said that young mice derive all of their nourishment from the mother for the first ten or eleven days after they are born, and thereafter appear to nurse as much and as often as the female will let them, a fact which undoubtedly accounts for the precise number of suckling young exerting a definite influence on the rate of growth of intrauterine embryos.

If only one or two young mice are suckling the development of eggs and embryos proceeds as though none were suckling, but if three or more young nurse the gestation period is lengthened, according to both Daniel ('10) and to the small amount of evidence on this point possessed by the present writer, approximately one day for each animal suckled. This observed fact is, however, very difficult to correlate with the series of embryos obtained from females suckling three to eight young and killed from fifteen to twenty-four days after parturition (table 2). These embryos show a variation in state of development which appears to vary neither with the number of young suckled nor with the number of embryos carried; in fact it would seem as though in one instance (female killed eighteen days post partum table 2) that the embryos, being as fully developed as in a non-suckling female of the same age post-partum, would have come to birth on the twenty-second day following the previous parturition in spite of there being three young suckling. Even supposing this had happened, were all the facts known such an exception would probably be explainable, since occasional females may reasonably be expected to possess amounts of nourishment far in excess of the average, and some litters of young may start eating grain at an earlier age than usual. More difficult of explanation are such cases as the embryos of a female killed seventeen days

TABLE 2

Data regarding all suckling white mice from which embryos were obtained from the fifteenth to the twenty-fourth day following parturition. Embryos labeled (small) would probably all have been absorbed

AGE IN DAYS POST-PARTUM	DEVELOPMENT EQUAL TO EMBRYOS OF NON-SUCKLING ♀s OF	NUMBER OF SUCKLING YOUNG	NUMBER OF EMBRYOS
	<i>days p. p.</i>		
15	8	8	11
16	12	4	8
17	14	5	2 large + 6 small
18	18	3	9
19	12	7	2 large + 4 small
20	8	6	5
21	8	5	9
21	12	8	9
22	8	?	10
23	12	7	9
24	14	4	6 large + 1 small

post-partum (table 2) which, if, as we have every reason to believe, they became implanted at the close of the fourteenth day post-partum must in the course of the three days following have undergone a development which in embryos in non-suckling females requires eight days to complete, and this in spite of there being five suckling young.

At the present time work is in progress with a view to explaining, if possible, these apparent contradictions and until that work is completed, which may not be for some time, it does not seem desirable to attempt any further analysis of the facts presented in table 2.

SUMMARY

1. The present work has brought together sufficient data with which to determine, within the possible error of one day, the age of all embryos obtained from non-suckling white mice.

2. Ovulation, fertilization, and the early cleavage of the eggs bear the same time relations to parturition and to one another in both suckling and non-suckling white mice except the former are much more apt to skip an ovulation period.

3. Implantation of embryos in the uterus occurs in non-suckling white mice on the fifth day following parturition (provided the female did not skip an ovulation cycle).

4. Implantation of embryos in the uterus occurs in suckling white mice, with 3 or more young, on the fourteenth day following parturition (provided the female did not skip an ovulation cycle). In these lactating females the blastulas lie free in the lumen of the uterus from the sixth to the fourteenth day post-partum due supposedly to the activity of the mammary glands.

5. The available material of stages following implantation in suckling females shows no evident correlation with either the number of nursing young or the number of embryos being carried. It also is impossible at present to reconcile the development of these embryos with the observed facts regarding the time of parturition in suckling mice.

6. The conflicting evidence from post-implantation stages in suckling females is at present being subjected to further study.

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ON THE POSTNATAL GROWTH OF THE BODY AND OF THE CENTRAL NERVOUS SYSTEM IN ALBINO RATS THAT ARE UNDERSIZED AT BIRTH

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Among the newborn young of various species of mammals there occasionally appear individuals that are undersized and have a very small weight at birth, although they are apparently normal in all other respects. Such individuals are very generally called 'runts' and they are usually discarded by breeders at birth, since it is the popular belief that they never attain normal size and that they are always sterile. Little is known of the true status of these animals, as few attempts have been made to rear them for the purpose of studying their growth processes and reproductive capacity.

In the course of an extensive series of breeding experiments with the albino rat a number of litters have been obtained in which one or more of the rats was very small at birth and weighed much less than the average birth weight for the young of the species, which is about 4.5 grams for the male and 4.3 grams for the female rat (King, '15 a). An examination of these litters some twenty-four hours after birth has shown, as a rule, that the very small individuals were dead, although all of the other members of the litter were alive and vigorous. Thus even under very favorable environmental conditions rats that are much below the average size at birth have little chance, apparently, of surviving even the first few hours of postnatal life; in a state of nature probably very few of them ever live to reach maturity.

In three of the litters examined the undersized young survived the seemingly crucial twenty-four period, and it was found possible to rear them with the other members of the litter and to study their growth in body weight. In order that the small

individuals might have every possible chance for subsequent growth, the young that were not of the same sex as the smallest member were discarded from each litter. This gave two series of female rats, one containing three and the other four individuals; and one series of four males. The rats in the first and those in the third series belonged to the twentieth generation of an inbred strain of albinos in which matings had been made only between brother and sister of the same litter in each generation; the rats in the second series were the offspring of an inbred female (nineteenth generation) and a stock male. For convenience litters with a parentage like that of the second series are designated as 'half-inbred' litters.

The rats in each series were weighed for the first time before they had suckled and then daily for one week. Thereafter the weighings were made at intervals varying from two to fifteen days until the rats were 150 days old when the experiment was ended. In an investigation of this kind it is impossible to obtain the exact body weights owing to the varying amounts of food in the digestive tract at the time that the animals are weighed. To minimize this source of error as much as possible all of the weight records were taken in the morning before the rats had received their daily ration.

Table 1 gives the birth weights of the rats belonging to the three series and also their later body weights at the different ages for which records were taken.

There was considerable variation in the birth weights of the rats in each of the three litters, as is shown in table 1, but in no series was the range of variation as great as that known to occur within the species (King, '15 a). In the first series the heaviest rat (no. 3) weighed but slightly more than the average weight for the female albino rat at birth, yet this weight is 77 per cent greater than the weight of rat no. 1 which had the smallest birth weight that has been found, as yet, in any female rat in our colony. The range of variation in the birth weights of the rats in the second series is much less than that in either of the other series, yet the weight of the heaviest member (no. 4) is 46 per cent greater than that of the smallest member. In the

Showing the body weights in grams, at different periods from birth to 150 days of age, of three series of albino rats

AGE IN DAYS.....		BODY WEIGHT IN GRAMS																							
Birth	1d	2d	3d	4d	5d	6d	7d	10d	12d	14d	17d	20d	25d	30d	40d	50d	60d	75d	90d	105d	120d	135d	150d		
Series 1 (Females)	1	2.6	3.0	3.4	3.9	4.5	5.1	5.8	6.6	8.9	12.3	14.5	17.2	19.0	28.0	46.4	55.0	76	84	106	113	123	132	133	145
	2	4.4	5.0	5.4	6.0	6.7	7.6	8.4	9.5	12.9	16.0	18.4	21.7	24.5	35.6	51.2	65.0	93	100	120	138	148	150	152	153
	3	4.6	5.2	5.6	6.5	7.4	8.4	9.3	10.6	14.5	17.2	20.0	23.4	26.8	37.3	54.7	62.0	88	97	127	137	149	153	155	158
Series 2 (Females)	1	3.1	3.4	3.8	4.5	5.3	5.9	6.6	7.2	8.8	10.5	12.0	14.7	18.0	19.9	31.6	45.4	55	58	79	110	112	114	124	128
	2	3.9	4.3	4.9	6.0	6.8	7.5	8.1	8.4	10.7	12.2	13.8	16.7	20.5	27.3	34.2	49.4	57	61	95	130	132	139	144	134
	3	4.4	5.0	5.3	6.5	7.4	8.1	8.6	9.3	11.4	12.8	14.5	17.4	21.6	25.9	37.8	52.9	63	69	103	129	136	143	146	149
	4	4.5	5.0	5.4	6.8	7.5	8.6	8.9	9.4	12.2	13.5	14.9	17.7	22.1	29.4	38.7	56.6	71	78	113	137	143	146	154	159
Series 3 (Males)	1	2.7	3.2	3.4	3.7	4.4	4.9	5.7	7.0	10.6	13.3	17.5	21.6	29.0	40.8	50.2	80.9	87	93	142	161	196	203	215	217
	2	3.4	4.0	4.6	5.3	6.0	7.0	8.4	10.4	16.1	19.9	23.7	29.7	34.1	44.9	54.0	83.3	101	111	162	192	223	236	256	263
	3	5.2	5.8	6.5	7.9	8.9	10.6	12.7	14.8	21.8	24.7	28.7	36.6	39.8	51.4	63.5	87.2	115	119	180	209	250	267	282	283
	4	5.3	5.9	6.8	8.0	9.4	11.1	13.4	15.9	22.2	24.8	30.2	36.9	44.1	61.9	73.3	101.3	120	125	182	205	238	250	270	272

third series the birth weights ranged from 2.7 grams to 5.3 grams, the largest individual being nearly twice as heavy as the smallest.

All of these rats followed the normal course of the growth in body weight, as already determined by Donaldson ('06), in spite of the very great differences in their birth weights. Increase in body weight was very rapid during the early days of postnatal life. The rate of growth dropped off somewhat abruptly at about thirty days, and again showed a marked decline at seventy-five days. After the rats reached ninety days of age the period of rapid growth was ended, and the rats gained relatively little in body weight up to 150 days of age when the weighings were discontinued. With few exceptions the body weights of all individuals fall within the range of variation in the body weights of stock albino rats of like age (King, '15 b, table 3). None of the rats that were unusually small at birth, therefore, could properly be considered as 'runts' when they became mature.

The rats that were undersized at birth never succeeded in attaining a body weight equal to that of the other individuals in the same series at any period of their growth, and at successive weighings the actual weight differences between the individuals that were small and those that were heavy at birth tended to increase. This was true for the individuals in each of the three series, irrespective of sex, as is shown by the data in table 1. At the end of 150 days the rats in each series still maintained the same order with respect to body weight that they had at birth, with the exception of rat no. 3 in the third series. This rat overtook its brother, which had a heavier birth weight, when it was ninety days of age and it subsequently kept the lead in body weight until the end of the experiment.

According to the 'standard' tables for the relation of age to body weight and to body length in the albino rat as given by Donaldson ('15), breeding females should have a body length of 193 mm. and a body weight of 186.1 grams when they are 150 days of age; the body length of a male albino rat of the same age should be 207 mm. and the body weight 218.7 grams. Records for the growth in body weight of a selected series of stock albino rats reared in The Wistar Institute animal colony under

environmental conditions similar to those under which the rats used in the present experiment lived (King, '15 b) show that for this group the average body weight of breeding females at 151 days of age is the same as that given by Donaldson, namely 186.1 grams; the average weight of the males of the same age is 244.8 grams, which is 26.1 grams above the computed weight for the male as given in Donaldson's tables.

The average body weight of all of the females used in this experiment was 146.7 grams, and that of the males was 258.7 grams, when the animals were 150 days old. The females, as a group, are too light in weight for their age, while the males are much too heavy, whichever of the above series of records is taken as a standard for comparison.

The fact that the females were either strictly inbred or half-inbred does not account for their small size, since in the strain of albinos from which these rats were taken inbreeding has increased rather than diminished the average body weight of both males and females (Popenoe, '16). Investigations made by Watson ('05) have shown that female rats that are allowed to breed are heavier at a given age than non-breeding females. It is probable that the low weight of these females is due, in some measure at least, to the fact that the rats were never mated (the stock females whose weights were given for comparison were all breeding animals). The relatively large size of the males in the third series can be attributed to the fact that the animals were from a selected inbred strain.

The average daily percentage gain of these rats in body weight during the period covered by the experiment is shown in table 2.

The percentage values for weight increase, unlike the weight data, show no definite order with respect to the birth weights of the individuals concerned. At some periods the rat which had the smallest birth weight shows a greater daily percentage gain in weight than any other member of the same series; at other periods the weight excess is in favor of the individual with the heavier birth weight (table 2).

If, instead of considering all of the percentage values in a given series, only the data for the two individuals that had the extreme

TABLE 2
Showing the average daily percentage weight increase of three series of albino rats (weight data in table 1)

		DAILY PERCENTAGE INCREASE IN BODY WEIGHT																							
RAT NO.		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	10-12 days	12-14 days	14-17 days	17-20 days	20-25 days	25-30 days	30-40 days	40-50 days	50-60 days	60-75 days	75-90 days	90-105 days	105-120 days	120-135 days	135-150 days	150 days over birth	
Series 1 (Females)	1	15.38	13.33	14.70	15.38	13.33	13.72	13.79	11.61	19.32	8.94	6.20	3.45	9.47	13.14	1.85	3.81	1.05	1.74	0.44	0.59	0.48	0.05	0.60	5476.92
	2	13.63	8.00	11.11	14.66	13.43	10.52	13.09	11.93	12.01	7.50	5.91	4.30	9.06	10.44	1.99	4.30	0.75	1.33	1.00	0.48	0.09	0.04	3377.27	
	3	13.04	7.69	16.07	13.84	13.53	10.71	13.97	12.68	9.31	8.13	5.66	4.84	7.83	9.32	1.33	4.19	1.02	2.04	0.52	0.58	0.18	0.09	0.04	3313.04
Series 2 (Females)	1	9.67	11.76	18.42	17.77	11.32	11.86	9.09	7.40	8.65	7.14	7.50	7.48	2.11	11.76	4.39	2.11	0.54	2.42	2.61	0.12	0.12	0.58	0.21	4029.03
	2	10.25	13.95	22.24	13.33	10.39	8.00	3.70	9.12	7.00	6.55	7.00	7.58	6.63	5.05	4.41	1.53	0.70	3.71	2.45	0.10	0.38	0.24	0.46	3335.89
	3	13.63	6.00	22.64	13.84	9.46	6.17	6.97	7.52	6.14	6.64	6.66	8.04	3.98	9.19	3.99	1.90	0.95	3.28	1.68	0.36	0.34	0.14	0.14	3286.36
	4	11.11	8.00	25.92	10.39	14.66	3.47	5.61	9.92	5.32	5.18	6.26	8.28	6.60	6.39	4.62	2.54	0.98	2.99	1.41	0.29	0.14	0.46	0.17	3433.33
Series 3 (Males)	1	18.51	6.25	8.82	18.91	11.36	16.32	22.80	17.14	12.73	15.79	7.80	11.41	8.14	4.60	6.11	0.75	0.69	3.51	0.89	1.45	0.24	0.39	0.06	7937.03
	2	17.64	15.00	15.21	13.40	16.66	20.00	23.80	18.27	11.86	9.54	8.43	4.93	6.33	4.05	5.40	2.12	0.99	3.06	1.23	1.07	0.39	0.56	0.19	7635.29
	3	11.53	12.07	21.53	12.65	19.10	19.81	16.53	16.43	6.65	8.09	9.17	2.91	4.51	4.70	3.73	1.90	3.53	4.11	0.71	1.30	0.45	0.31	0.02	5323.07
	4	11.32	15.25	17.64	17.50	18.08	20.72	18.65	13.20	5.85	10.88	7.39	6.50	8.13	3.68	3.82	1.84	0.42	3.04	0.84	1.04	0.34	0.53	0.05	5032.07

birth weights are compared, the results obtained seem consistent enough to be significant. In the total of 69 records for the three series, 42, or nearly two-thirds, show that the daily percentage weight increase is greater for the individuals with a low birth weight than for those with a heavy birth weight. In other words, regardless of sex, rats that are very small at birth tend to grow more rapidly than do rats that have a heavy birth weight, although their actual body weights are always less at any given period.

On computing the percentage increase in body weight at 150 days over the birth weight for the various individuals it was found that the rats that were small at birth had gained a greater amount than had the rats that were heavy at birth. For each series, as shown in the last column in table 2, these percentage values stand in inverse order to that of the birth weights, with the one exception in the second series (rat no. 4).

The results of this investigation are in accord with those obtained by Dunn ('08) in her study of the weight increase in a 'group' of seven albino rats (three males and four females) which had very unlike body weights when they were fourteen days old. Dunn found that, with one exception, the order relation of the weight at fourteen days of age was maintained until the end of the experiment; the rats having the heaviest initial weights were also the heaviest at sixty-six days of age when the weighings were discontinued. The lighter rats, on the other hand, while putting on less absolute weight, had gained at the end a greater percentage of their original weight than had the individuals with the heavier initial weight.

In addition to the undersized young which, as shown above, are capable of developing into adults which are only slightly below normal, a litter sometimes contains individuals of a much lower grade to which the term 'runt' properly applies. In these individuals, which apparently are indistinguishable from the other young at birth, the normal action of the growth factors is inhibited from the very beginning of postnatal life by unknown constitutional causes, not by environmental conditions. When the young rats are old enough to leave the nest the runts can

easily be distinguished from the other members of the litter, not only because of their very small size, but also because of their slower movements and apparent lack of normal vitality. Runts grow slowly for a certain time, but no matter how favorable the external conditions, they never exhibit normal vigor and they are always dwarfed and stunted in their body growth. In such animals growth is not merely retarded, as it is in the case of rats experimentally stunted (Hatai, '07; Osborne and Mendel, '14), but it is permanently checked at an early age. Several attempts have been made in our colony to increase the size of runts by special feeding, and to breed them for the production of a dwarfed race of rats. Only a few litters could be obtained from such stock, and these contained a very small number of young which were puny from birth and which died at an early age. The reproductive powers of these animals are apparently never developed in a normal way, as the males rarely mate and most of the females are sterile.

All of the rats used in this study were killed at the end of 150 days, the body weights and body lengths determined, and the brains and spinal cords removed and weighed. This was done in order to ascertain whether the central nervous system in adult rats that were small at birth bears the same relation to body weight and to body length as that found in adult individuals that were of average size, or above, at birth.

Table 3 gives the body lengths of the individuals, the observed weights of the brains and of the spinal cords, and the brain and cord weights corrected according to table 68 in 'The rat: data and reference tables' (Donaldson, '15) which gives the computations for the 'standard' weights of the central nervous system in albino rats of various body lengths. In addition to the above data, table 3 shows the percentage deviations of the observed weights of the brains and of the spinal cords from the corresponding standard weights.

It is evident, from the percentage values given in the fifth and in the eighth columns of table 3, that the observed weights of the brain and of the spinal cord in all of the rats are considerably below the standard weights for these organs in animals

TABLE 3

Showing the body lengths, with the observed and the 'standard' weights for the brain and for the spinal cord, of the albino rats whose weight data are given in table 1

	RAT NO.	BODY LENGTH IN MM. (150 days)	OBSERVED BRAIN WEIGHT	STANDARD BRAIN WEIGHT FOR BODY LENGTH	PER CENT DEVIATION OBS. BRAIN WEIGHT FROM STANDARD	OBSERVED CORD WEIGHT	STANDARD CORD WEIGHT FOR BODY LENGTH	PER CENT DEVIATION OBS. CORD WEIGHT FROM STANDARD
Series 1 (Females)	1	183	1.461	1.773	-17.5	0.455	0.524	-13.1
	2	185	1.611	1.782	-9.5	0.473	0.532	-11.0
	3	182	1.679	1.768	-5.0	0.499	0.520	-4.0
Series 2 (Females)	1	175	1.511	1.735	-12.9	0.418	0.492	-15.0
	2	184	1.616	1.778	-9.1	0.467	0.528	-11.5
	3	192	1.653	1.814	-8.8	0.485	0.560	-13.4
	4	188	1.643	1.796	-8.5	0.475	0.544	-12.6
Series 3 (Males)	1	198	1.697	1.849	-8.1	0.529	0.556	-4.8
	2	210	1.765	1.903	-7.2	0.579	0.603	-3.9
	3	217	1.955	1.933	+ 1.1	0.631	0.630	+ 0.2
	4	212	1.999	1.911	+ 4.6	0.669	0.611	+ 9.4

of like body length, except in the case of the two largest males where the observed weights slightly exceed the standard weights. That the weights of the central nervous system in all individuals of a given litter, having the same sex and about the same body weight, should deviate from the standard weights in the same direction was not an unexpected result. In the rat variation within the litter unit is usually in the same direction and much less than that in the general population as regards body weight (Jackson, '13; King, '15 b), and doubtless this rule holds for the central nervous system and other organs as well. In every series, as shown in table 3, the rats with the smallest birth weights are the ones whose brain and cord weights show the most marked deviations from the standard weights, regardless of their body length and body weight with which the weight of the central nervous system is, as a rule, closely correlated. Thus, in the first series, female no. 1 had a body length of 183 mm. and a body weight of 145 grams while female no. 3 was shorter and heavier,

yet in the former individual the brain was 17.5 per cent and the cord 13.1 per cent below the corresponding standard weights; in the latter individual the brain and cord weights were only about 5 per cent less than the standard weights. All of the rats in the second series had cord weights that showed relatively greater deviations from the standards than did the brain weights; the lowest weight for both brain and cord being found in the rat that had the shortest body length and the smallest body weight (no. 1). The most interesting result is found on comparing the records for the rats belonging to the third series. The brains of the two rats that had birth weights much lower than the average birth weight (nos. 1 and 2) were each about 8 per cent less than the standard and the cord weights showed a minus deviation of some 4 per cent from the standard, yet in body measurements rat no. 1 was below and rat no. 2. was above the average for stock males of like age. The brain and cord weights of their brothers, each of which was unusually heavy at birth, were above the computed standard weights for the central nervous system in animals of like body length.

That the postnatal growth of the central nervous system is influenced to some extent by the factors that determine the size of a rat at birth seems to be a conclusion warranted by the analysis of data given above.

SUMMARY AND CONCLUSIONS

The results obtained in this investigation seem to indicate that the undersized individuals which are sometimes found in a newborn litter of rats are not necessarily 'runts' in the generally accepted use of that term. Some of these small individuals, as shown above, attain an adult size that not only is within the normal limits of variation in the body weights of standard stock rats of like age, but may even exceed the average body weight of a large number of stock animals (rat no. 2, series 3).

All rats in a litter are not born with a like capacity for growth, as the data in table 1 indicate, and even when environmental conditions are as favorable and as uniform as it is possible to make them, individuals having unlike birth weights show marked differences in their rates of growth from birth to the adult state.

In the cases studied the individuals having a small birth weight seemed to possess a very great capacity for growth from the very beginning of postnatal life. Female no. 1 of the first series increased 2 per cent more in body weight during the first twenty-four hours after birth than did either of her sisters; for the same period the gain in body weight of the two smallest males in the third series was over 6 per cent more than that of their brothers. In the second series, however, the weight increase for the first day was greater for the rats that were heavy than for those that were small at birth.

With an early acceleration in the rate of growth there is seemingly correlated an early cessation in the extent of growth, as in the adult state individuals that were undersized at birth are always smaller than the other members of the same litter. On the other hand, rats with a heavy birth weight tend to grow more slowly at first than do the smaller individuals, but they continue to grow for a longer time and eventually reach a greater size. This rule seems to apply to females as well as to males.

Not only does body weight at birth indicate the probable capacity of the individual for subsequent growth, but it also indicates the probable size of the central nervous system, since rats that are undersized at birth tend to have a much smaller central nervous system when they become mature than do other rats. The factors, whatever their nature, that determine the body size of a rat at birth seem to have a marked effect on the subsequent postnatal development of the individual, influencing the ultimate body weight as well as the size of the central nervous system.

A very small weight at birth indicates that a rat has a handicap in its organization that environment, however favorable, cannot overcome. Such animals, although they appear vigorous and healthy during their growth period and after reaching the adult state, are unquestionably sub-normal in regard to the size of the body and of the central nervous system. If allowed to breed these rats would probably produce young having a weaker constitution than their own, and from such stock one would ultimately get 'runts' and an increasing tendency towards sterility that would soon bring disaster to the colony.

Judging from the results obtained in this study a newborn litter of rats may contain individuals of three kinds as regards their inherent capacity for body growth. As a rule, only young rats having a normal birth weight and a normal capacity for growth are found in a small or medium sized litter produced by a female rat in good physical condition. Occasionally rats are born which have a very small birth weight, and in these individuals, if they are able to survive, the growth capacity is lessened to some extent but not sufficiently to prevent them from being classed as 'normal' after they have reached maturity. If a litter is very large, or if the mother is not in good physical condition during the gestation period, some of her young may be born with their growth capacity so impaired that it is impossible for them to grow beyond a certain stage. These individuals are true 'runts' and, fortunately, they are lacking in reproductive vigor as well as in growth capacity so that they are usually unable to reproduce their kind and so prove a menace to the colony in which they live.

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MOUNTING SPECIMENS UNDER PETRI DISHES AND CLOCK GLASSES

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Since obtaining a suitable cement for the sealing of square museum jars, the mounting of thin sections of pathological and anatomical specimens under Petri dishes and clock glasses has been made both easy and cheap. Several years ago these mounts attracted a great deal of attention, being put on the market as paper weights, etc., but were gradually adopted for the exhibition and display of museum specimens. The method, however, being patented was expensive and beyond the reach of the average museum; besides it was necessary to send your material for mounting to the manufacturer. In 1906 Dr. Hutchinson of the Royal Victoria Hospital, Montreal, read a paper before the British Medical Association at Toronto on this method; but unfortunately he did not have a suitable cement and the process was slow and laborious.

METHOD

The fluid used for mounting should be brought to a boil in the same dish that is to be used for mounting and allowed to stand over night, or until cool, to get rid of as much air as possible. The dish should be deep enough to come well up over the mount to allow of easy manipulation of both Petri dish and specimen. Get a Petri dish of suitable size to hold the specimen so that when the sheet of glass which is to form the cover of the mount is in position it will not quite touch it. Great care must be taken that the Petri dish or clock glass fits perfectly on the base and does not rock.

In placing the Petri dish in the mounting fluid, do so without causing any air bubbles. When the dish and fluid are ready, wash the specimen in several changes of the same fluid that you are mounting in, to get rid of any loose particles or dirt. In the last change of fluid, work out all air and remove quickly to the Petri dish, face downwards, again eliminating air bubbles. A small piece of looking-glass in the bottom of the mounting dish is very convenient, as by tipping the Petri dish over and on its side slightly, any bubbles under the specimen may be seen.

The specimen now being in position, put on the base or cover and remove from the mounting-dish, holding it firmly so that it cannot slip

and admit air while turning the mount right side up. Over the junction of Petri dish and cover pour hot cement¹ to the thickness of about one quarter of an inch, and allow the mount to stand on a flat surface for a few days, or until the cement is perfectly adherent to both Petri dish and cover. If after several days it is desired to finish the mount, remove any bubbles of fluid between the cover and the cement with a very hot knife, working the knife to the outer edge of the base. This must be carefully done, and the knife kept hot enough so that the cement will be kept liquid and not be drawn away from the Petri dish. All the fluid must be removed from under the edge of the Petri dish in this way.

The best results are obtained, however, by allowing the mount to stand for several weeks when most of the excess fluid will work out by itself. I usually mount several dozen specimens at a time and let them stand until they are ready to finish. If any air bubbles should happen to get under the edge of the Petri dish they will have to be worked into the mount in the same way that fluid is removed, only work your knife inwards instead of outwards.

Even with the greatest care air is often retained in the specimen itself, and only detected after the mount has stood for several days. Should the bubbles be very small they will quite frequently be absorbed by themselves; if not, shake the mount until they are all in one large bubble, and resubmerge in mounting fluid which must be heated as hot as you can comfortably stand your hand in. When the cement has become pliable enough so that it is possible to move the Petri dish with the fingers, insert the point of a sharp knife between the dish and base, gently forcing them apart and allowing a little fluid to enter. The mount must then be tilted on its side to bring the air bubble to the opening, where it will escape when you remove the point of the knife; then press the Petri dish up against the base, expelling all superfluous fluid. Close the hole by pressing the soft cement together with the fingers.

Air bubbles may have to be removed several times depending on the specimen. Sections of lung give the most trouble. When sure that there are no more bubbles in the mount and that all fluid has been removed from between the cement base and the Petri dish, coat the cement over to the thickness of about one-eighth of an inch with refined asphalt, being careful that it does not burn; I usually melt it in a tea-spoon over a Bunsen burner. When the asphalt has been all applied, reheat the whole with a very hot knife and apply a bezel ring which must be heated red hot, and pressed down into the cement so that the lower edge will rest upon the base. Clean with gasolin and polish with bon ami soap.

The Petri dishes used are manufactured specially from 2 to 3 mm. thick, as the ordinary ones sometimes break with the expansion and

¹ See Muir and Judah, Sealing of museum jars. Bulletin 5, Inter. Assoc. Med. Mus., page 87.

contraction of the fluid. The bezel rings which are used to give a finished appearance to the cement can be made out of any metal that will stand being heated red hot. They are not, however, absolutely necessary, as you can use a sheet of cardboard with a hole cut in the centre and passe partout the edges. Dr. Higgins of the Experimental Farm, Ottawa, has a very convenient cardboard case into which he slips the mount. If a bezel ring is to be used, the base or cover should be made out of plate glass with the edges bevelled.

Clock glasses are inferior to Petri dishes for this method because they magnify and distort the specimen.

The following list of sizes have been found to answer all requirements. While 36 in number they only require ten different sized bezel rings and plate glass bases, a desired advantage when the glass ware is made to order.

Sizes (Outside measurements)

SIZE NO.	WIDTH	HEIGHT	SIZE NO.	WIDTH	HEIGHT
	<i>cm.</i>	<i>cm.</i>		<i>cm.</i>	<i>cm.</i>
1	18	3	19	8	2.5
2	18	2	20	8	2
3	18	7	21	8	1.5
4	16	3	22	8	1
5	16	2	23	6	2.5
6	16	1.5	24	6	2
7	14	3	25	6	1.5
8	14	2.5	26	6	1
9	14	2	27	4	2.5
10	14	1.5	28	4	2
11	12	3	29	4	1.5
12	12	2.5	30	4	1
13	12	2	31	3	2
14	12	1	32	3	1.5
15	10	2.5	33	3	1
16	10	2	34	2	2
17	10	1.5	35	2	1.5
18	10	1	36	2	1

ANNOUNCEMENT

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THE EFFECT OF HYPOPHYSECTOMY IN THE EARLY EMBRYO UPON THE GROWTH AND DEVELOPMENT OF THE FROG

A PRELIMINARY REPORT

P. E. SMITH

From the Anatomical Laboratory, University of California

TEN FIGURES

The extirpation of the hypophysis in the adult frog has not given uniform results. Caselli ('00) and Gaglio ('02) who reported no changes following hypophysectomies were followed by Boteano ('06) who reported a neuromuscular asthenia in the operated animals. Houssay ('10) came to the conclusion that the removal of the gland was followed by death. Adler ('14) burned out the hypophysis of a 20 mm. *Rana temporaria* larvae with the electric cautery. Out of the 1200 operated animals three were found to have been hypophysectomized, not, however, without great injury to the surrounding soft parts, particularly the brain. In not one of those three animals did hind legs develop beyond a small bud, and transformation did not take place, the specimens remaining as neotonic tadpoles.

This work was commenced in the Spring of 1914, repeated in 1915, and again in 1916, *Diemyctylus torosus*, *Rana pipiens*, and *Rana boylei* being successively used. In this paper the results obtained with the California yellow-legged frog, *R. boylei* are reported. Shortly after the closure of the medullary plate, Kopsch's stages d-e, was found to be the size in which the hypophysial invagination could be most successfully removed. About 200 larvae of this stage were operated upon. In specimens of this size the hypophysis was successfully removed in over 60 per cent of the operated animals. Approximately 30 per cent of those animals in which the gland was extirpated did

not give reliable results in the rate of growth as the mouth was wholly or partially removed thus interfering with feeding. Unoperated animals and those in which the ablation of the gland was unsuccessfully attempted were available for checks.

The operation is a simple procedure. The hypophysial invagination can be accurately determined from the pit that it early forms or from its location between the protuberance of the forebrain and the stomadeum, which is just forming. This epithelial ingrowth was removed with some neighboring epithelium. The wound healed within three hours in most cases, less than 1 per cent of the larvae disintegrating after the operation. The operated animals and checks were kept in boiled water for five days and then transferred to a frog tank where they were in an essentially normal environment.

The rate of growth in the hypophysis-free animals has been slower than in the checks. The larger hypophysectomized animals averaged smaller in size than the larger checks, the averages of the two showing a noticeable difference. On June 6 the operated but not hypophysectomized animals had an average length of 40 to 43 mm., the hypophysis-free animals averaging 33 to 35 mm., a ratio constant throughout their growth. The ratio of body to tail length is the same in the two classes, the difference in size being uniform for all parts of the animal. The tail fin did not show an increased width or pleating in the hypophysectomized animals as reported by Adler ('14).

In activity the two classes of animals showed no marked differences. The hypophysectomized specimens were perhaps slightly more alert, darted more quickly, and consequently were more difficult to capture with the pipette than were the checks.

The resistance of the hypophysectomized animals was greater than that of the checks. Towards the close of the experiment the animals were attacked by disease, none reaching the adult stage. The normal specimens succumbed more rapidly to this infection than did the hypophysectomized ones. Some of the intrinsic factors which induce growth of legs and transformation were lacking in the abnormal specimens as will be shown later. The absence of these factors may well be conducive to a greater

hardiness in an animal when compared to the normal tadpole in which the usual rapid changes are taking place.

Differences in color began to be noticeable before a length of 15 mm. was reached, and from then on the contrast in pigmentation between the hypophysectomized animals and the checks was striking. Those animals without hypophyses were characterized by a light grayish appearance; however, the dorsal side was more pigmented than the ventral (figs. 7, 10). These are referred to as albinos. The checks were a brown-black color often showing a mottling (figs. 8, 9). This color difference was more noticeable over the body than on the tail, but was evident in both regions and was the most striking feature up to the time of the appearance of the hind legs in the checks. Sections show that these pigment differences are referable chiefly, if not solely, to the condition of the epidermis. Counts of the melanophores of corresponding areas in the albinos and in the checks show that the number of these cells, in the epidermis, are reduced in the former. Further the melanophores of the albino specimens contain fewer pigment granules than do those of the checks and thus have a distinctly lighter appearance. The melanophores are equally expanded in the two types, consequently, the lighter color of the albinos cannot be due to the contracted condition of the chromatophores but must be referred, in part, to the reduced number of melanin granules in the pigment cells of the epidermis. In addition to this the free pigment granules which form a distinct zone in the superficial layer of the epidermis in the normal checks are much reduced in number in the albino specimens (figs. 5, 6). It is surprising that in the albinos the deeper or subcutaneous pigment is present in as great a quantity as in the normal animals, if not greater. The amount and distribution of the retinal pigment seem to be identical in the two.

Another important feature was the inhibition in growth of the hind legs of the operated animals. There was only a slight retardation in the time of appearance of the hind leg buds, normally, appearing when the tadpole has reached a length of 25 to 27 mm. In the albino, averages show that the hind limb buds appear when the larvae are from 26 to 28 mm. in length.

From this state on, however, the hind limbs in an hypophysectomized animal grew but little if at all, although the animal's length increased at a rate but slightly under the normal. The accompanying table shows the increase in length of the hind legs in relation to total length for the albinos and for the checks. (See also figs. 7, 8).

Average rate of growth in millimeters in terms of total length, of the hind legs of the checks and the albinos

HYPOPHYSECTOMIZED ANIMALS		CHECKS	
Total length	Hind leg length	Total length	Hind leg length
26	barely visible	25	barely visible
28	0.1	28	1.0
30	0.1	30	2.0
35	0.1	35	3.0
37	0.12	38	4.0
		40	5.0
		45	9.0

Only one exception to the rule that no hind legs grew on albinos was found. A 36 mm. albino had hind legs 4.2 mm. long when killed. The above is in accord with Adler ('14) who found that removal of the hypophysis in a 20 mm. stage inhibited the growth of the hind legs.

Examination of sections of albino and normal animals shows striking differences in the endocrine glands. The sectioned hypophysectomized animals show no trace of the anterior lobe of the hypophysis. That part of the floor of the diencephalon which normally abuts against the hypophysis, rests upon the floor of the cranium (fig. 2). This apparently demonstrates conclusively that the entoderm has not the intrinsic power to form a hypophysis. If it enters into the formation of the gland at all it must be considered as a tissue inclusion which became changed through its adaptability into glandular parenchyma, a conclusion previously drawn by the writer, Smith ('14). The infundibulum shows some structural modifications when compared to the checks, although the saccus vasculosus, as far as determined, appears to be normal. In the checks that region of the diencephalon which rests against the pars glandularis is

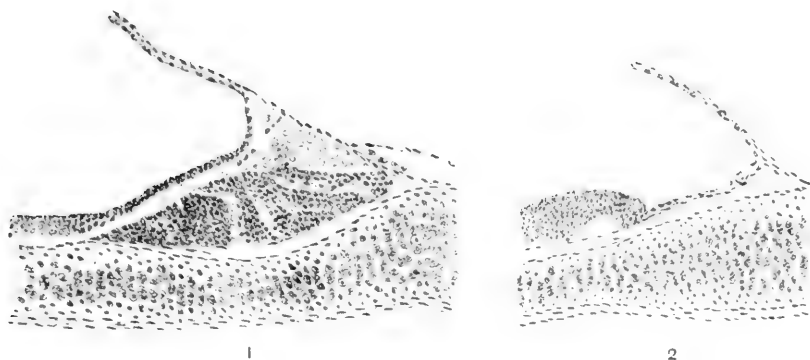


Fig. 1 A section through the hypophysial region of a 38 mm. normal tadpole. $\times 100$.

Fig. 2 A section through the hypophysial region of a 37 mm. albino. Note the much reduced pars nervosa. $\times 100$.

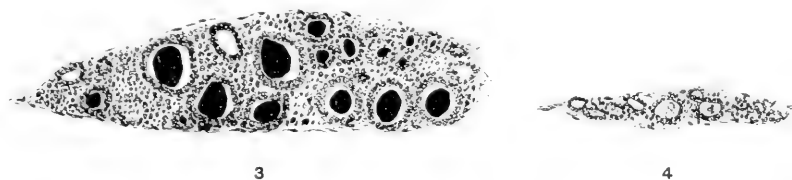


Fig. 3 A sagittal section through a lobe of the thyroid of a 38 mm. check. $\times 100$.

Fig. 4 A sagittal section through a lobe of the thyroid of a 37 mm. albino. $\times 100$.

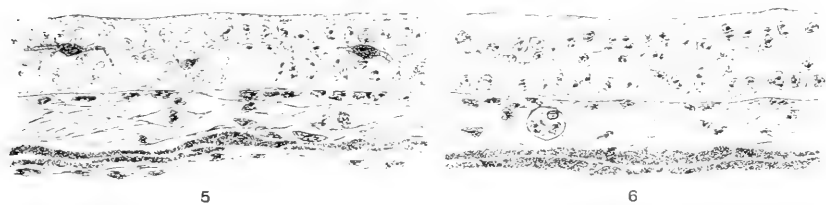


Fig. 5 A section through the epidermis, in the mid-brain region, of a normal 39 mm. check. The pigment granules are indicated by dots. $\times 200$.

Fig. 6 A section through the epidermis, in the mid-brain region, of a 38 mm. albino. A faint melanophore in the left part of the figure. $\times 200$.

of considerable thickness, that is, in addition to the ependyma there is a rudimentary pars nervosa. Caudad to this the wall is formed almost entirely of ependyma. The pars nervosa is reduced throughout most of its extent to an ependymal layer in the hypophysectomized animals. There may be a small localized thickening but nothing to correspond to the normal animal (figs. 1, 2).

The thyroid shows marked modifications in the albinos. In the accompanying table the size of one lobe of the thyroid of a normal 38 mm. tadpole with 4.0 hind legs and of a 37.0 mm. albino with 0.1 mm. hind legs is given.

<i>Size in millimeters of one lobe of the thyroid</i>			
<i>38 mm. check</i>		<i>37 mm. albino</i>	
Length.....	0.6	Length.....	0.21
Width.....	0.3	Width.....	0.15
Thickness.....	0.16	Thickness..	0.04



Fig. 7 Photograph of an albino. $\times 2$. Note the very small hind limb bud.
Fig. 8 Photograph of a normal tadpole. Figures 7 and 8 were photographed on the same plate $\times 2$.
Fig. 9 Photograph of a normal tadpole. $\times 2$.
Fig. 10 Photograph of an albino. $\times 2$.

The above table shows that the thyroid of the albino is approximately one-third normal size. The contrast is even more striking when the compactness and character of the parenchyma is noted. A sagittal section through the thyroid of a 38 mm. check shows on an average 12 to 15 vesicles, many of which are largely distended with colloid, the parenchyma of the whole gland being compacted together. A sagittal section through the thyroid of a hypophysectomized 37 mm. specimen shows 6 to 8 atrophied vesicles containing but a slight amount, or no colloid, and with large spaces between the vesicles. The cells making up the vesicles of the former are cuboidal and protoplasmic-rich, in the latter little but the nuclei remain (figs. 3, 4). The results from experimental feeding of thyroid by Gudernatsch and other workers suggests that the non-development of the hind legs in the albinos is due not to the hypophysis but rather to the failure of the thyroid. In this connection the 36 mm. albino with 4.2 mm. hind legs, mentioned above, is of interest. Sections of this specimen show that the hypophysis was completely ablated but that the thyroid is normal. This specimen thus gives additional evidence that the retarded development of the hind legs must be referred to the thyroid and not to the hypophysis. Also the reduction in pigment is not due to the atrophy of the thyroids. The modifications of the thyroid obtained by Adler ('14) were similar but less striking.

An examination of a large number of male and female albinos and checks has, as yet, failed to show any constant variation from the normal in the sex glands of the hypophysectomized animals. The sex glands of the albinos although varying considerably apparently do not exceed the limit of variation met with constantly in the normal animals. This conclusion stands in contradiction to the results previously adduced by the author and to the results of Adler ('14) in the hypophysectomized tadpole and to the conclusions of Hahn ('12) in the tadpole with hypertrophied hypophysis as well to the results obtained in mammals by pituitary feeding, notably that of Goetsch ('16).

The writer wishes to express his appreciation to Dr. H. M. Evans for his generous aid.

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ANOMALIES IN LOBATION OF LUNGS WITH REVIEW OF LITERATURE

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FIVE FIGURES

Judging from the literature, abnormal lobation of the lungs is relatively rare.

Lindsay ('10) divided the abnormalities in the lobation of the lungs into two classes: those in which the normal number is decreased and those in which the number of lobes is increased. The former is due either to a deficiency of the lobes themselves, or to a deficiency of the fissures, which normally separate the lobes. The latter is due to an increase of lobes, or to an increase in fissures. Complete absence of lobation in definitely formed lungs is rarely if ever found, though its homologue is to be found in the Orang, with two lungs each existing as single lobes.

Rokitansky ('61) showed that arrests of development may occur and that this may lead to complete absence or great deficiency of one or both lobes. This arrest may be so early that the lungs can scarcely be observed as small round bodies situated at the ends of the bronchi. This condition is generally due to contraction of the volume of the thorax.

Pontif ('60) in 'Virchows Archives' recorded a case in which the right bronchus was connected with an ovoid body, which was imbedded in gelatinous tissue and filled the right half of the thorax.

According to Lindsay, cases of class two, that is those which have an excessive number of fissures, are occasionally encountered, and are probably the most common form of abnormality. They do not appear to present any regularity, and lack the interest which is attached to accessory lobes. There are two

groups of accessory lobes. One which is of considerable developmental interest, is composed of completely isolated masses of pulmonary tissue formed between the diaphragm and the base of the left lung; occasionally on the right side such masses are found even in the abdominal cavity. Other masses containing arteries, veins, nerves, and bronchial tissue, but devoid of bronchi, are attached to the oesophagus, aorta or other mediastinal structures by a pedicle. These masses are apparently quite functionless. Two such cases are recorded by Vogel ('99), in each of which he found a deficiency in the bronchial tree. Simpson ('99) described a case of a deficient bronchial tree found in a foetus.

In the cases with additional fissures there is a normally placed lung presenting an excessive number of lobes. These abnormalities are usually very definite in their position, and occur more commonly on the right side.

Wrisberg ('77), who was the first to notice an accessory lobe in the human lung, recorded a most interesting and unique case of an accessory lobe on the left side produced by the left azygous vein; i.e., the superior intercostal vein which preserved its foetal condition and opened into the left innominate vein.

Chiene ('76), described a pear-shaped supernumerary lobe, lying between the upper lobe of the right lung and the bodies of the dorsal vertebrae, having its origin from the angle formed by the junction of the upper lobe with the root of the lung. The supernumerary lobe was separated from the upper lobe of the lung by a double fold of the pleural membrane, which descended vertically for seven centimeters from the apex of the thoracic cavity where it was continuous with the pleura costalis. It enclosed in its free border the vena azygous, and formed the outer wall of the cul-de-sac, in which the supernumerary lobe was contained. The left side of the chest was normal; both sides were healthy.

E. W. Collins ('88), recorded a case of an accessory lobe immediately above the posterior part of the root in the angle between it and the upper portion of the right lung. This accessory lobe was somewhat pyriform in shape, with a broad pedun-

cular attachment. In all, Collins was able to collect seven cases of accessory lobes in human lungs.

A. E. Maryland ('90) described abnormalities in lobes of three lungs. The first was a right lung with no indications of a middle lobe, but a development of a third, or accessory one, on the inner side of the lung. The second was a left lung with a subdivision of the upper lobe. The third was a right lung with an incomplete separation of a normal middle lobe. Maryland states: "Cases of more than four right lobes and three left lobes are exceedingly rare."

Patterson ('09-'10) described a condition of two additional lobes. One of these was above the root, and separated from the upper lobe by a fissure. This accessory lobe was enclosed within a pleural pouch, which contained the vena azygous. The second additional lobe was below the root, and between the upper and middle lobes.

Case I of the present specimens presents features entirely different from those hitherto described. It was obtained in the dissecting room during the term of 1915-1916 from a male subject, aged sixty-one. The cause of death as given on the death certificate was dementia. No clinical history was obtainable. Besides the anomalous condition of the lungs, there was a persistent thymus, and a number of anomalous arteries. On gross inspection the lungs presented no pathologic lesions.

The left lung apex-base measured 22 cm., dorso-ventrally 20 cm. The normal fissure (*F3*), which separated the superior from the inferior lobe, was in its normal position, starting at the junction of the antero-inferior border 25 cm. from the apex, and running obliquely upward and backward, dividing the superior and inferior portions completely.

The superior portion, however, presented two other fissures, as shown in the diagram, thus dividing this portion into three more or less distinct lobes.

Fissure number one (*F1*) started at the anterior border 7 cm. below the apex, and ran horizontally backward on the antero-lateral surface of the lung for 6 cm. The depth of the fissure was on an average 1.5 cm.

Fissure number two (*F2*) started at the antero-median border 17 cm. below the apex and ran upward and backward for 9 cm. This fissure extended through the lung tissue, completely separating the middle from the inferior division of the superior lobe. Neither of the two mentioned fissures extended as far as the main fissure, which separated the superior from the inferior lobe.

Three distinct divisions of the superior lobe were evident from an antero-lateral view. The upper division (*L1*) was pyramidal in

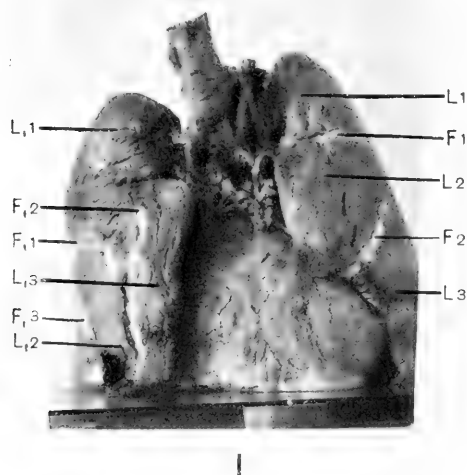


Fig. 1 Anterior aspect

shape, forming the apex. It measured 8 cm. antero-posteriorly, and 7 cm. from apex to base.

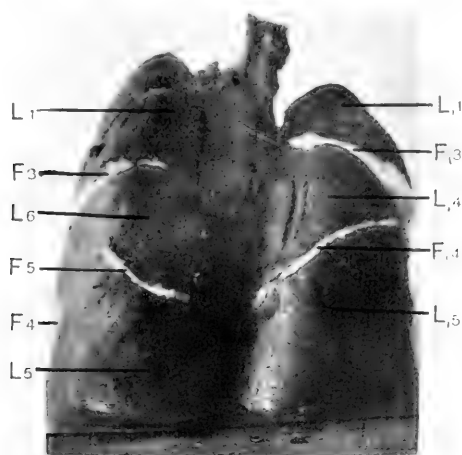
The middle portion (*L2*) was wedge-shaped, wider on the anterior border than on the posterior. It measured 9 cm. on the anterior border between the first and second fissures, and 17 cm. antero-posteriorly.

The lower division (*L3*) is lingual in shape. This division measured 6 cm. on the anterior border, and 21 cm. antero-posteriorly.

The inferior lobe presented two more supernumerary fissures as did the superior lobe.

Fissure number four (F_4) started 5 cm. antero-inferiorly from the supero-inferior fissure and ran parallel with the above fissure for a distance of 10 cm. This fissure also extended through the lung tissue separating the upper from the postero-inferior divisions.

Fissure number five (F_5) was on the lateral surface, extending obliquely, superiorly and inferiorly, and incompletely dividing the postero-lateral from the postero-inferior divisions.



2

Fig. 2 Posterior aspect

The inferior lobe, which was triangular, also presented three fairly distinct divisions.

The upper division (L_4), which is oblong in shape, having a small tongue-like projection on the anterior border, ran obliquely upward, and backward, following the direction of the supero-inferior, or great fissure. This division measured 5 cm. antero-inferiorly, and 19 cm. antero-posteriorly.

The postero-inferior division (L_5) was more or less quadrangular in shape. It measured 8 cm. on the superior border and 12 cm. on the inferior border, i.e., at the base.

The postero-lateral division (L_6) was irregular in outline. The upper border was made by the supero-inferior fissure, and

the lower by a separate fissure between this division and the postero-inferior division.

The right lung apex-base measured 19 cm., and dorso-ventrally 18 cm. In this lung the fissures were deeper, and went through the lung tissue, completely dividing the lung into distinct divisions. The normal fissure ($F'3$), which divided the apex lobe and middle lobe from the inferior, or base lobe, was a little more irregular than the corresponding fissure on the left lung;

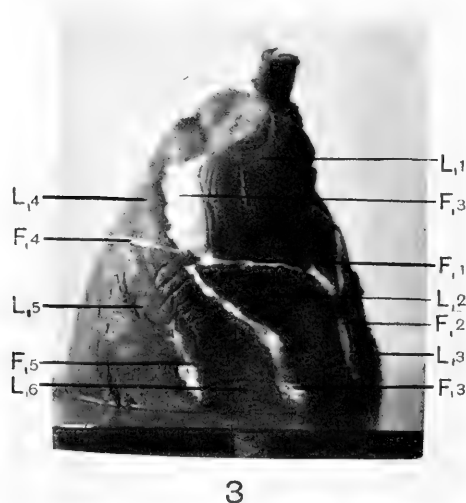


Fig. 3 Right lung: lateral view

and ran obliquely upward and backward, dividing the lung into an upper and lower division.

The upper division presented an irregular outline from a lateral view. There were two large fissures; one which normally divided the apex lobe from the middle lobe, and the other a supernumerary fissure dividing the middle lobe into two. There was also a small fissure in the apex lobe.

Fissure number one ($F'1$), which divided the apex lobe from the middle one, started 10 cm. from the apex laterally, midway between the apex and the base.

Fissure number two ($F'2$), which divided the middle lobe into two divisions, ran vertically for a distance of 8 cm., starting from the base and running toward the apex.

The apex lobe ($L'1$) was quadrilateral in shape, having the upper border narrower than the base and measuring 9 cm. apex-base, and 8 cm. antero-posteriorly.

The inferior division ($L'2$) of the upper lobe was lingual in shape, extending obliquely upward and backward 13 cm. in its long direction, and measuring 4 cm. apex-base.

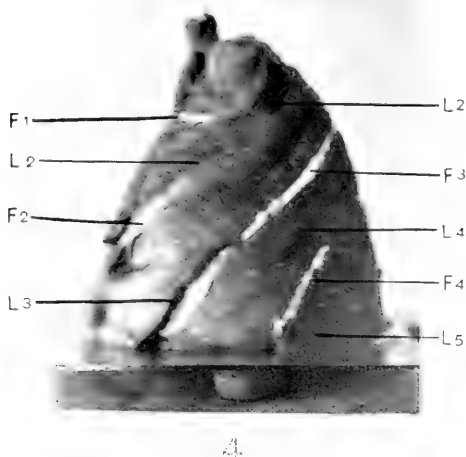


Fig. 4 Left lung: lateral view

The inferior division ($L'3$) of the upper half of the lung was elongated in outline measuring 4 cm. in antero-posterior direction, and 13 cm. apex-base.

The lower half of the right lung was triangular in shape, having two clearly visible fissures which ran through the lung tissue and divided this portion of the lung into three distinct lobes. There are also two smaller fissures in the inferior border of the lung, merely forming small tongue-like lobules.

The fourth fissure ($F'4$) started 10 cm. from the apex midway between apex-base, running downward and backward for a dis-

tance of 14 cm., and completely separating the upper from the lower division of this half of the right lung.

The fifth fissure ($F'5$) started from the base, or inferior border, and ran vertically upward for a distance of 10 cm. It extended through the lung tissue, dividing this inferior half into two divisions, a posterior and an antero-median.

The superior division ($L'4$) of this inferior half of the right lung was triangular in shape, measuring 13 cm. on its inferior

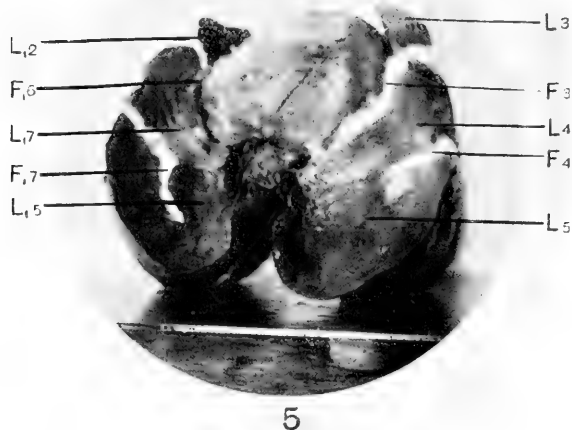


Fig. 5 Base

border, 8 cm. on its superior border, and 11 cm. on its posterior border.

The postero-inferior division ($L'5$) was quadrilateral in outline, measuring 8 cm. on its shortest border and 14 cm. on its longest, or posterior border.

The antero-median division ($L'6$) had the form of an elongated triangle, measuring 11 cm. on the anterior border, 6 cm. on the median posterior border.

At the base of the right lung was a very irregularly shaped division distinctly separate from the rest of the lung as shown

in the diagram. This basal division (*L* 7) measured 10 cm. in the long direction and 6 cm. in the short direction. This division was separated from the rest of the lung by two distinct fissures (*F'* 6-7) which started at the postero-inferior border, and ran for 9 cm. anteriorly and backwards.

Case II was obtained in the dissecting room during the term 1915-1916, from a female subject, aged thirty-eight.

The cause of death as given on the death certificate was 'septic meningitis.' No clinical history was obtainable.

The left lung apex-base measured 20 cm., dorso-ventrally 18 cm. The right lung apex-base measured 18 cm., dorso-ventrally 17 cm.

The left lung presented upon examination one accessory lobe, one accessory lobule, and three accessory fissures in the inferior portion of the left lung.

The right lung presented upon examination two accessory lobes and two accessory fissures. The first accessory lobe was in the upper portion of the inferior division of the right lung. The other accessory lobe was a basal lobe and was identical with the basal lobe seen in the right lung in Case I.

X-Ray¹ pictures of Case II show separate bronchi going to each of the main lobes including the accessory lobes, as may be seen by referring to the plates.

X-Ray views of Case I were unsuccessful on account of a poor bismuth injection.

From the foregoing description it is evident that these specimens show no distinct azygous lobe, which is the common accessory lobe described. These lungs retain their normal shape; but they present, besides the normal fissures, a number of accessory fissures which divide the lungs into distinct accessory lobes.

Complete absence or deficiency of one or both lobes may be due as Rokitsansky points out to arrests of development as contraction of the volume of the thorax. Supernumerary lobes have been variously explained. Lindsay ascribes a slight adhesion of the lungs to the thoracic wall as the cause of the super-

¹ Stereoscopic X-ray plates show the separate bronchi as described. Reduced prints fail to show the necessary details and, therefore, are not reproduced here.

numerary lobe, or possibly an undue curvature of the embryo, so that the Venae Cavae, as it bent down to a position at right angles to its original position, instead of slipping behind the pleura and lung, dragged down a fold of the former and deeply notched the latter.

Fischer remarks that it is difficult to conceive of the azygous lobe without an already preëxisting anomalous course of the azygous, and makes no attempt to explain the phenomenon.

Collins explains the azygous lobe as a persistent foetal condition of the left azygous vein.

All previous attempts to explain the origin of the supernumerary lobes apply only to cases in which there is a definite azygous lobe. The anomalous course of the azygous vein has constricted off a portion of the lung tissue, thereby forming a new lobe. This explanation does not apply to cases in which there are no azygous lobes and in which, however, there are other supernumerary lobes, as the azygous lobes are not in reality true lobes. A true lobe as applied to the lung indicates a separate bronchus. Whether the azygous lobe has a separate bronchus, or not, has not been stated in their descriptions.

Considered from the viewpoint of their origin, a constricted portion—such as the azygous lobe—is not a separate entity, but a part of the mother lobe from which it has separated.

The formation of lobes of the lungs has first been studied by Aeby and was worked out by Narath. Aeby defines lung lobe as follows: "A true lobe is never supported by more than a single bronchus and therefore includes no portion of the stem bronchus." Soon after the formation of the first lateral bud in the embryo, each bud becomes marked out upon the surface of the mesodermal anlage of the lung. Before development of lateral bronchi, the surface of this anlage is smooth. Later it becomes almost mulberry-shaped, and secondary elevations are then formed by the budding of the bronchial bud which it contains. The process goes on until the surface becomes covered with fine granules. These disappear with further growth of the lung—only the first formed furrows persisting normally.

Accessory lobes may then be due to a retention of the foetal condition in which not only the primary divisions persist, but on account of the rather slow growth of the bronchi, the secondary conditions persist—that is to say, accessory lobes are not new formations, but represent a stage in the normal development of the lung.

Accessory fissures dividing the lobe into a number of lobules—as exists in the present specimens—may have been due to folds of splanchnopleura which have been carried down and caused the formation of a groove, lined with pleura, or they may have been caused by incomplete obliteration of the secondary divisions, which are produced in the mulberry stage and which normally disappear.

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ANOMALOUS RENAL VESSELS AND THEIR SURGICAL SIGNIFICANCE

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NINE FIGURES

Abnormalities of the renal arteries occur more frequently, perhaps, than anomalies of any of the other larger vessels. In view of the enormous number of investigations of the different structures of the kidneys recorded in the literature on the subject, it seems strange that only scanty information exists concerning the actual course of the larger blood vessels, and their relations to the pelvis of the kidney. The normal, as well as the abnormal, arrangement of the renal vessels at the hilum is known; the microscopic picture of the vessels in the cortex and pyramids are likewise thoroughly familiar to every student; but as to the form of the pelvis, and the actual course and distribution of the larger vessels around its walls, very vague ideas still prevail.

Professor Thane states that irregularities of the renal arteries are met with in about 25 per cent of cases, and that the most common irregularity is the presence of an additional vessel in about 20 per cent.

Young and Thompson report four cases of anomalous renal arteries: the first is that of multiple renal arteries and malposition of the right kidney; the second that of multiple renal arteries, malposition, and malformation of both kidneys; the third that of a horseshoe kidney with multiple renal arteries; the fourth that of multiple renal arteries, two of which were on the left side, two on the right. In the last case there were also multiple spermatic arteries, and two renal veins on the right side, while the left side was normal.

Irregularities in renal vessels have also been mentioned by McAllister ('83) who found anomalous renal arteries in 43 per cent of the cases examined. Levings ('12) reported two cases of anomalous renal vessels going to the lower poles. Harvey ('14) described a case of multiple renal arteries.

The specimens of the present description were obtained in the dissecting room through the help of Dr. E. B. Trovillion, Instructor in Anatomy, University of Colorado; and from autopsies through the courtesy of Dr. R. C. Whitman, Professor of Pathology, University of Colorado. We were able to examine, in all, 33 cases of which 22, or 73 per cent possessed anomalous renal vessels.

Case I. The kidneys were normally placed in the abdominal cavity. Both were somewhat smaller than normal. The right showed two small cysts on its anterior surface. It lacked the normal large renal artery, but possessed instead two renal arteries of equal size, which were almost as large as the normal vessel should have been. The lower artery arose from the ventro-lateral portion of the aorta, 6 cm. above its bifurcation, and passed to the lower pole where it divided into two vessels, 2 cm. before it entered the kidney substance. The second artery arose from the aorta 6 cm. above the lower one, and split into two vessels 2 cm. before it reached the kidney substance. There were also two large renal veins, closely accompanying the arteries; one arising from the upper, the other from the lower pole of the kidney. Both veins emptied into the vena cava.

The left kidney possessed three renal arteries and one renal vein. The lowest renal artery arose from the left ventral portion of the aorta 4 cm. above the bifurcation of the aorta, and supplied the lower pole of the kidney. The second artery arose from the aorta 6 cm. above the first, and supplied the upper pole. The third arose 1 cm. above the second, crossed it and entered the kidney at the hilum. One large renal vein arose from the kidney at the hilum.

Case II. Both kidneys were larger than normal, and in the proper position. The right possessed one renal artery and one renal vein. The left kidney, however, possessed three main renal arteries, and one renal vein. The first renal artery, which seemed to be the normal vessel, arose from the ventro-lateral aspect of the aorta just opposite the origin of the superior mesenteric artery. The second renal artery arose 1 cm. above the first. This was a long slender artery having a tortuous direction, and entered the kidney 4 cm. above the inferior border. The third artery was the smallest, and arose from the aorta just lateral to the second. This artery broke up into three smaller branches, all of which supplied the upper pole of the kidney. The veins were normal.

Case III. This presented two normally placed kidneys, the right being smaller than the left. The right kidney presented upon examination two renal arteries and one renal vein. The lower renal artery arose 2 cm. above the bifurcation of the aorta from its ventral aspect, and entered the kidney at the lower pole 1 cm. above the inferior border. The upper renal artery, which was the normal one, arose from the dorsal aspect of the aorta 11 cm. above the bifurcation of the latter, and, after running a tortuous course, entered the kidney at the hilum. The left kidney was normal. Two spermatic veins emptied into the left renal vein.

Case IV. This presented upon examination two normally placed kidneys. The right possessed two arteries; the normal one arose from the aorta, and entered the kidney at the hilum; the anomalous one arose from the ventro-lateral aspect of the aorta 5 cm. above the normal renal vessel, and entered the upper pole after it had split into two branches.

The left kidney also possessed two arteries; the normal one coming from the ventro-lateral aspect of the aorta just opposite the normal right renal artery, and entering the kidney at the hilum; the accessory renal artery branching from the superior mesenteric, and entering the upper pole of the left kidney 3 cm. below the superior border. The veins in both kidneys were normal.

Case V. This subject presented two normally placed kidneys of normal size. The right kidney contained a small anomalous artery arising from the aorta, and supplying the upper pole. The left kidney was normal, as were also the veins.

Case VI. This presented two normally placed kidneys. The right contained four large renal arteries and two large renal veins. The first renal artery arose from the ventro-lateral aspect of the aorta 3 cm. above the bifurcation, and entered the kidney on the posterior surface of the lower pole 3 cm. above the inferior border. The second and third arteries took origin from the aorta at the ventral surface as a common large branch 1 cm. above the bifurcation but immediately divided into two rather large branches, which entered the kidney at the hilum. The fourth artery was a long slender one coming from the coeliac axis, and entering the kidney at the upper pole 4 cm. below the superior border. There were two renal veins present; a large one entering the vena cava, and another somewhat smaller one also entering the posterior aspect of the vena cava. The left kidney possessed one accessory artery arising directly from the aorta and entering the kidney substance at the lower pole.

Case VII. This was a case of a right kidney with a small accessory artery arising just above the normal renal artery, and entering the kidney at the upper pole 3 cm. below the superior border.

Case VIII. In this case the left kidney was somewhat larger than normal, and contained three large renal arteries, one of which divided into three smaller branches. The first artery arose from the aorta, and entered the kidney at the lower pole, 4 cm. above the inferior

border. The second artery was the largest of the three. It arose from the aorta above the first, and entered the kidney at the hilum. The third artery arose from the aorta above the second, and divided into three smaller branches all of which supplied the upper pole. Two large renal veins were present which arose from the hilum and entered the vena cava.

Case IX. Both kidneys were normal in size and position. On the right side one large arterial trunk sprang from the aorta. This divided into two rather long slender branches after it had continued its course for 2 cm. Each of the two branches further divided into two other branches, thus four arteries entered the kidney: two at the upper pole 3 cm. below the superior border; the other two at the lower pole 4 cm. above the inferior border. Connecting the main renal trunk with the aorta was a plexus of vessels of varying sizes. This plexus, covering the walls of the aorta, appeared to be a persistence of the embryonic periaortic plexus. There was one renal vein which came from the hilum and entered the vena cava.

The left kidney possessed two large and two small arteries. The first, which seemed to be the normal one, arose directly from the aorta and entered the kidney at the hilum. The second artery, which was a long slender vessel, came from the aorta and entered the anterior surface of the kidney 4 cm. below the superior border. On the left side two small arteries came from a plexus which resembled the periaortic plexus. On this side, these small arteries entered the kidney substance, while on the right side the plexus merely connected the large renal vessels with the aorta.

There were also two large renal veins, two smaller veins and a plexus of still smaller veins. This plexus connected the larger veins with the vena cava. The largest vein, which seemed to be the normal one, came from the hilum of the kidney and entered the vena cava. The second large renal vein, which was a long slender one, connected the left spermatic vein with the anterior surface of the kidney. The plexus not only connected the vena cava with the large renal veins, but also formed an anastomosis between this venous plexus and the arterial plexus, so that the venous and arterial blood had a chance to mix.

Cases X, XI, XII, and XIII. These four subjects had kidneys which were similar in most respects. There were two renal arteries arising from the aorta and entering the hila of the right and left kidneys in each case. The veins were normal.

Cases XIV, XV and XVI. These three cases possessed small accessory arteries arising from the aorta and entering the poles of the kidneys. In cases fourteen and fifteen the accessory arteries entered the lower poles, and in case sixteen the small arteries entered the upper pole.

Case XVII. This case possessed four arteries to the right kidney and three to the left, all of which arose directly from the aorta. There were also two renal veins from the left kidney, both of which arose from the hilum and entered the vena cava.

Case XVIII. The right kidney possessed two small renal arteries coming directly from the aorta and entering the kidney at the hilum. The left kidney as well as the veins were normal.

Case XIX. Both kidneys possessed three renal arteries rather small in size, which entered the kidney at the hilum. The veins were normal.

Case XX. The right kidney was normal. The left possessed two renal arteries, both of which were branches of the aorta, and entered the kidney at the hilum. The veins were normal.

Case XXI. The right kidney possessed two renal arteries, one of which entered the superior, the other the inferior pole. The left kidney possessed three renal arteries, which arose from the aorta as separate branches, and entered the kidney at the hilum. The veins were normal.

Case XXII. The right kidney possessed three renal arteries, arising from the aorta and entering the kidney from the hilum. The left kidney possessed two renal arteries, one arising from the aorta and entering the kidney at the hilum, the other arising from the coeliac axis and entering the kidney at the superior pole 2 cm. from the upper border. The veins were normal.

The embryological origin of the renal arteries has not yet been satisfactorily explained. The explanation as here offered is after Keibel and Mall.

His ('80) first observed multiple branches of the aorta supplying the mesonephros in 7 mm. embryos. A more extended account of them has been given by Broman. At first, when the Wolffian bodies are relatively small, the mesonephric vessels are correspondingly small. They come from the middle portion of the aorta (2nd to 8th thoracic). At the end of the first month the mesonephros reaches its greatest development. It receives many direct branches from the aorta at levels cranial as well as caudal to the original ones. In 8 mm. embryos there are twenty mesonephric arteries on each side (8 cervical to 12th thoracic segments). The last vessels to appear grow out from the region between the first and second lumbar segments. These are destined to persist as the remainder atrophy. There are then on each side—in maximo—thirty vessels distributed throughout the entire mesonephric area. At first these are entirely distributed to the mesonephros, but later also supply the reproductive glands, suprarenal bodies, metanephroi, and diaphragm. These new regions of distribution prevent their com-

plete degeneration when the mesonephros disappears. A variable number of them persist as phrenic, suprarenal, renal, accessory renal, internal spermatic, accessory spermatic arteries, and as the rami ad lympho-glandulas and ad sympatheticum. The first mesonephric arteries found in embryos 5.3 mm. arise from the lateral surface of the aorta, and pass horizontally to the urogenital fold, reaching the malpighian corpuscles, and terminating in them with an enlargement which usually assumes a spherical shape. Later a network of vessels occupies the place of the enlargement. This network is also connected with the

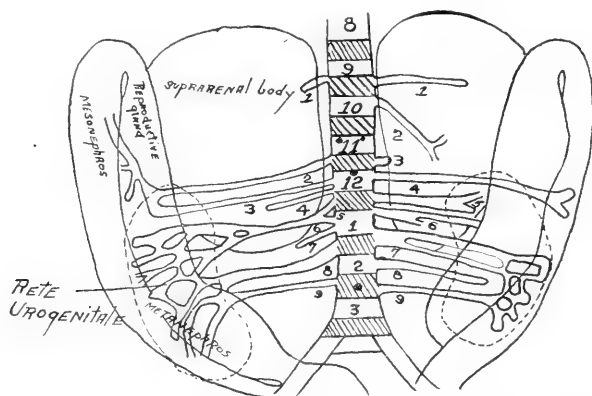


Fig. 1 Mesonephric arteries in human embryo of 18 mm. greatest length. Circles on anterior surface of aorta indicate origin of coeliac, superior and inferior mesenteric arteries (after Keibel and Mall).

posterior cardinal vein. Case IX is an example of the persistence of this anastomosis. With increasing age the arteries continually recede into the lumbar segments disappearing from the thoracic ones.

The arteries are divided into three groups by the suprarenal body: the cranial group, which is dorsal to the suprarenal (1-2); the middle group, whose vessels pass through the suprarenal (*R.* 3-4, *L.* 3-5); and the caudal group whose vessels pass over the ventral side of the suprarenal body (*R.* 5-6, *L.* 6-9). The mesonephric arteries (5-9) situated in the angle formed by the reproductive gland ventrally, the mesonephros laterally, and the

metanephros dorsally, form a network: the rete arteriosum urogenitale. The mesonephros, reproductive gland, and the metanephros are supplied with arterial branches from this network, thus making these organs independent of single branches for their blood supply. Should one or several roots degenerate, neighboring arteries can take their places. In the above diagram, for instance, the second mesonephric artery has divided into an ascending and descending branch; the ascending one supplies the entire upper half of the mesonephros and the reproductive gland, a region that in the young embryo receives its blood from several mesonephric arteries belonging to more cranial segments. The occurrence of this network at once explains why all persistent arteries that arise from the roots of this network show, within certain limits a variability in the points of their origin from the aorta. Each of the nine to eleven remaining mesonephric arteries may become an internal spermatic artery, since all supply the reproductive glands. This explains the frequently observed multiplicity of these arteries, and the not infrequent difference in the place of origin of the right and left ones.

The renal arteries are not new formations, as some have claimed, but each is formed from a mesonephric artery. The kidney climbs upward to the mesonephric artery and as soon as sufficient blood supply is assured cranially, the caudal branches separate from it. When the kidney has acquired its definitive position it possesses several arteries, and of these one becomes greatly enlarged to form the definitive artery, while the others either degenerate, or persist as accessory renals. The definitive renal artery is either the last vessel of the second group, or first of the third group. The relations of both groups, i.e., of the second to the suprarenal artery, and of the third to the internal spermatic, explain the variation in which the renal artery arises from a suprarenal or from an internal spermatic. In the first case it may be the principal stem; in the latter, only an accessory renal. The relations between the urogenital rete and metanephros show how the accessory renal arteries may develop, and explain their varied relations to the kidney. Accessory renal

arteries from the first group will be branches of the superior suprarenal, and must pass over the dorsal surface of the kidneys. These consequently first reach the kidney on its dorsal surface and there penetrate its cortex. Those from the second group will be branches of either the middle or inferior suprarenal, and will reach either the hilus or the kidney or the medial edge above this. Those from the third group may enter the hilus at the medial edge below it, or on the ventral surface of the caudal half of the organ. Should a caudal branch be retained, it will be drawn upwards by the migration of the kidney. This condition explains why such an artery may cross the principal stem or another accessory.

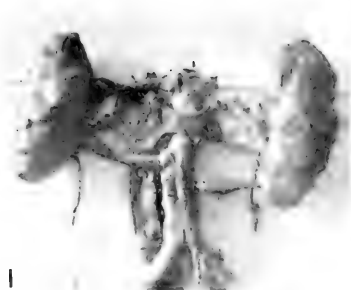
Anomalous renal vessels are not only interesting from a purely scientific point of view, but are also of very great significance from a clinical and surgical standpoint.

The Mayos in 20 out of 27 cases operated upon by them for hydronephrosis, found anomalous blood vessels. The obstruction in each case was caused by the blood vessel crossing the uretero-pelvic juncture. The vessels passed to the lower pole of the kidney, and varied from the size of a knitting needle to that of the radial artery.

Levings, in a report of four cases on which operations were performed, found anomalous arteries which crossed the ureter, and to which condition he attributed the clinical symptoms of the patients. He also reported post-operative improvement in every case.

Rupert in 1913 found anomalous renal vessels in 35 out of 50 cadavers studied. In every case the kidneys were normally placed, and of normal size and shape. He concluded that the usual percentage given is too low, and that anomalous renal veins, while not as common as the arteries, do occur, and that on account of the thinness of their walls and lack of pulsations they increase the hazards of kidney operations.

It is very evident from the specimens at hand that a number of these arteries might be overlooked if it were necessary to remove or examine any of these kidneys. In view of the fact that anomalous renal arteries are important it is rather strange



that their existence has been neglected in surgical anatomy teaching. As Rupert points out, the most common text books of anatomy and surgery make but brief mention of this condition, and some do not even refer to it at all.

The accepted percentage of 20 to 25 as given by Quaine and Gerrish is evidently too low. Senator as recently as 1905 made the statement that "Reduplications of one or both renal arteries is a rare condition and may be dismissed." Rupert found 35 cases or 70 per cent of anomalous renal vessels, while in this present investigation there are 22 cases out of 33, or 73 per cent. In all of these cases the kidneys were normally placed and the anomalous arteries were so arranged that they would easily complicate surgical procedures.

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SIZE AND LENGTH RELATIONS OF THE RIGHT AND LEFT TESTES OF PIGEONS IN HEALTH AND DISEASE

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The right ovary undergoes an early and more or less complete atrophy in most species of birds. Etzold ('91) has shown that in the sparrow the left testis is larger than the right. Firket ('14) and Swift ('15) have shown that in the chick embryo there were more primordial germ cells in the left gonad, and that this gonad is there also distinctly larger than the right. Allen ('07) found that the sex cells were unequally distributed to the two gonads of the turtle, the left receiving most. In this form only 24-70 per cent of the sex cells ever enter the gonads. Our own accumulation of data on the size and length relations of the two testes of young and adult pigeons show a very decided predominate number of larger right testes; and also a distinct difference in shape of the two glands—the left though actually smaller in size is usually absolutely longer than the right. Changes in the size relation in birds dead of certain diseases—particularly tuberculosis—and in hybrids are also suggested by our data

The meaning of this pronounced inequality in the distribution of the primordial germ cells which is plainly associated with a larger left embryonic gonad, and the finding in adults of two groups of birds of a marked and nearly constant larger gonad, but this a different gonad in the two cases, is by no means clear. But, whatever this may mean, it is probably a situation of importance to the theory of sex. We present our present data then with the confession that on the main points the meaning is not clear, but with the conviction that they are not less valuable because of our present inability to clarify the puzzling situation, and hopeful that the data may stimulate the further

accumulation of facts from enough forms, and of such varied kinds, as may lead to a better understanding of the embryonic and adult inequalities of the sex glands of birds.

An examination of our data has shown that the measurements of glands of healthy birds should be grouped apart from those dead of disease; and those of pure species should be separated from hybrids. The justification of these separate groupings will appear later.

The relative size of the sex glands in healthy common pigeons

The weights of 31 pairs of testes from healthy common pigeons are recorded in table 1. In 27 of these pairs the left testis was the smaller; in 4 the left was the larger. In two or three of these latter cases—12, 15 (22?)—the disparity of the two glands is so great as to make it clear that the smaller gland was wholly abnormal. In healthy common pigeons the right testis is larger than the left in a high proportion of cases.

Size relations of the testes of common pigeons dead of disease

In tables 2 and 6 the weights of 9 pairs of testes are given. In 7 of these the left gland was the smaller. It was larger in two instances; in one of these irregular cases, the smaller gland was again quite abnormally proportioned in reference to its larger associate

Size relations of testes of pure species, healthy and dead of disease

The testes of only 5 healthy birds of pure species (dead of cold, exposure, accident) are included in table 6. In all of these cases the right testis was the larger.

The data for 46 individuals of pure species dead of disease are available. In table 2, 9 of the 10 individuals listed had larger right testes; the tenth had the two glands of equal size. Eleven further comparisons are supplied in table 3. Of these, 7 right testes are larger, 2 are smaller, and 2 are the size-equivalents of the left. Table 6 gives the data for 30 additional pairs. Of these, 7 of *St. risoria* all had larger right testes; 2 of *T. orientalis* both had larger right testes; 13 of *Spil. tigrina*—mostly not

mature birds had, 5 larger, 5 smaller, and 3 equivalent right testes. Four miscellaneous birds here had 2 larger and 2 smaller¹ right testes.

TABLE I
Weight of right and left testes of healthy common pigeons

NO	DATE	WEIGHT	PER CENT OF DIFF.	NO	DATE	WEIGHT	PER CENT OF DIFF.
1	April 5	R = 0.885 L = 0.515	-71.8*	17	April 9....	R = 1.115 L = 1.015	-9.9
2	April 5.....	R = 1.475 L = 0.845	-74.6	18	July 5.....	R = 1.010 L = 0.750	-34.7
3	April 5.....	R = 1.185 L = 0.990	-19.7	19	July 5.....	R = 1.220 L = 1.375	+12.7
4	April 5.....	R = 1.055 L = 0.820	-28.7	20	July 5.....	R = 1.140 L = 0.970	-17.5
5	April 7.....	R = 1.410 L = 0.765	-84.3	21	July 5.....	R = 1.158 L = 0.765	-51.4
6	April 7.....	R = 1.190 L = 0.975	-22.1	22	July 5.....	R = 0.370 L = 0.720	+94.6
7	April 7.....	R = 1.260 L = 1.000	-26.0	23	July 9.....	R = 0.855 L = 0.800	- 6.9
8	April 7.....	R = 1.235 L = 0.945	-30.7	24	July 9.....	R = 1.643 L = 1.030	-59.5
9	April 7.....	R = 1.280 L = 1.225	- 4.5	25	July 13.....	R = 0.571 L = 0.540	- 5.7
10	April 9.....	R = 1.075 L = 0.900	-19.4	26	July 13.....	R = 0.820 L = 0.631	-29.9
11	April 9.....	R = 1.025 L = 0.710	-44.4	27	July 16.....	R = 1.820 L = 1.500	-21.3
12	April 9.....	R = 0.025 L = 0.715	+2760.0	28	July 18.....	R = 1.600 L = 0.536	-198.5
13	April 9.....	R = 1.460 L = 1.390	- 5.0	29	July 20.....	R = 0.051 L = 0.040	-27.5
14	April 9.....	R = 1.010 L = 0.720	-40.3	30	July 20.....	R = 1.390 L = 1.085	-28.1
15	April 9.....	R = 0.275 L = 1.425	+418.2	31	July 21 (juv.)	R = 0.0004 L = 0.0003	-33.3
16	April 9.....	R = 1.125 L = 0.500	-125.0	Left smaller in 27; larger in 4.			

* In calculating percentage differences in these tables the smaller gland is considered as equal to 100 per cent.

¹ In both of these cases where the right testes weighed less than the left it will be seen that both testes were quite small—so small as perhaps to raise a question as to the reliability of the weights.

Size of testes in healthy specific hybrids

In tables 4 and 5 the data for 30 healthy young hybrids are given. The very small gonad size of most of these young birds

TABLE 2
Weights of right and left testes of various pigeons (classified) dead of disease

NO.	DATE	WEIGHT	PER CENT OF DIFF.	NO.	DATE	WEIGHT	PER CENT OF DIFF.
1. Common pigeons							
32	July 28.....	R = 0.122 L = 0.460	+277.0	34*	September 28	R = 0.037 L = 0.034	-8.8
33*	September 1.	R = 0.580 L = 0.415	-39.8	35	December 14.	R = 1.300 L = 0.850(?)	-52.9
2. Blond and white wing doves and their hybrids							
Hybrids, (specific)				Pure			
36*	June 10.....	R = 0.417 L = 0.305	-36.7	42*	April 9.....	R = 0.045 L = 0.025	-80.0
37*	June 27.....	R = 0.050 L = 0.035	-42.9	43*	April 17.....	R = 0.060 L = 0.050	-20.0
38*	July 23.....	R = 0.032 L = 0.046	+43.8	44*	September 26	R = 0.055 L = 0.055	0.0
39	September 12	R = 0.765 L = 0.705	-8.5	45*	October 25...	R = 0.068 L = 0.056	-21.4
40	October 17..	R = 0.0053 L = 0.0067	+26.4	46	October 25..	R = 0.046 L = 0.033	-39.3
41*	October 31...	R = 0.090 L = 0.090	=0.0	47*	October 28...	R = 0.030 L = 0.027	-11.1
3. Other hybrids (specific, ex. 51, 52 = gen.)							
48	August 28....	R = 0.845 L = 0.600	-40.8	51*	September 18.	R = 0.580 L = 0.510	-13.7
49	June 10.....	R = 0.190 L = 0.160	-18.8	52	September 23.	R = 0.040 L = 0.031	-29.0
50	August 28....	R = 0.133 L = 0.115	-15.7	53*	September 27.	R = 0.010 L = 0.012	+20.0
4. Other pure species							
54*	August 26....	R = 0.040 L = 0.022	-81.8	56	October 31...	R = 0.465 L = 0.445	-4.5
55*	November 10	R = 0.033 L = 0.023	-43.5	57*	November 9..	R = 0.015 L = 0.013	-15.4

* Tuberculosis found.

TABLE 3.

Weights of testes of doves (classified) dead of disease March 29 to November 25, 1915

NO.	DATE	WEIGHT	PER CENT OF DIFF.	NO.	DATE	WEIGHT	PER CENT OF DIFF.
Pure species				Specific hybrids			
59*	March 29	$\left\{ \begin{array}{l} R = 0.012 \\ L = 0.018 \end{array} \right.$	+50.0	76*	May 1	$\left\{ \begin{array}{l} R = 0.030 \\ L = 0.031 \end{array} \right.$	+ 3.3
60*	April 1	$\left\{ \begin{array}{l} R = 0.008 \\ L = 0.005 \end{array} \right.$	-60.0	77*	July 23	$\left\{ \begin{array}{l} R = 0.262 \\ L = 0.212 \end{array} \right.$	-23.6
61*	April 2	$\left\{ \begin{array}{l} R = 0.105 \\ L = 0.105 \end{array} \right.$	= 0.0	78*	July 30	$\left\{ \begin{array}{l} R = 0.040 \\ L = 0.032 \end{array} \right.$	-25.0
62*	April 16	$\left\{ \begin{array}{l} R = 0.022^1 \\ L = 0.060 \end{array} \right.$	+172.7	79*	August 22	$\left\{ \begin{array}{l} R = 0.076 \\ L = 0.077 \end{array} \right.$	+ 1.3
63	September 2 . . .	$\left\{ \begin{array}{l} R = 0.158^2 \\ L = 0.146 \end{array} \right.$	- 8.2	80	August 29	$\left\{ \begin{array}{l} R = 0.605^2 \\ L = 0.454 \end{array} \right.$	-33.3
64*	September 5 . . .	$\left\{ \begin{array}{l} R = 0.032 \\ L = 0.020 \end{array} \right.$	-60.0	81*	September 19 . .	$\left\{ \begin{array}{l} R = 0.188 \\ L = 0.152 \end{array} \right.$	-23.7
65	September 11 (Juv.)	$\left\{ \begin{array}{l} R = 0.003 \\ L = 0.002 \end{array} \right.$	-50.0	82*	September 28 . .	$\left\{ \begin{array}{l} R = 0.026 \\ L = 0.026 \end{array} \right.$	=0.0
66*	September 14 . . .	$\left\{ \begin{array}{l} R = 0.023 \\ L = 0.023 \end{array} \right.$	= 0.0	83*	October 7	$\left\{ \begin{array}{l} R = 0.025 \\ L = 0.021 \end{array} \right.$	-19.0
67*	September 29 . . .	$\left\{ \begin{array}{l} R = 0.138 \\ L = 0.116 \end{array} \right.$	-18.9	84*	October 19 (Juv.)	$\left\{ \begin{array}{l} R = 0.007 \\ L = 0.006 \end{array} \right.$	-16.6
68*	October 3	$\left\{ \begin{array}{l} R = 0.045 \\ L = 0.030 \end{array} \right.$	-50.0	85*	October 24	$\left\{ \begin{array}{l} R = 0.030 \\ L = 0.030 \end{array} \right.$	- 0.0
69*	November 11 . . .	$\left\{ \begin{array}{l} R = 0.082 \\ L = 0.067 \end{array} \right.$	-22.4	86*	November 21 (Juv.)	$\left\{ \begin{array}{l} R = 0.015 \\ L = 0.020 \end{array} \right.$	+33.3
Generic hybrids				87*	November 22 . . .	$\left\{ \begin{array}{l} R = 0.014 \\ L = 0.016 \end{array} \right.$	+14.3
70*	April 2	$\left\{ \begin{array}{l} R = 0.140 \\ L = 0.125 \end{array} \right.$	-12.0	88	November 25 . . .	$\left\{ \begin{array}{l} R = 0.018 \\ L = 0.015 \end{array} \right.$	-20.0
71*	May 30	$\left\{ \begin{array}{l} R = 0.320 \\ L = 0.280 \end{array} \right.$	-14.3	Common pigeons			
72	June 20	$\left\{ \begin{array}{l} R = 0.082 \\ L = 0.076 \end{array} \right.$	- 7.9	89	April 28	$\left\{ \begin{array}{l} R = 1.260 \\ L = 1.035 \end{array} \right.$	-21.7
73*	July 16	$\left\{ \begin{array}{l} R = 0.300 \\ L = 0.298 \end{array} \right.$	- 0.7	90*	August 25	$\left\{ \begin{array}{l} R = 0.098 \\ L = 0.087 \end{array} \right.$	-12.6
74*	August 2	$\left\{ \begin{array}{l} R = 0.012 \\ L = 0.010 \end{array} \right.$	-20.0	91	April 5	$\left\{ \begin{array}{l} R = 1.105^3 \\ L = 0.990 \end{array} \right.$	-11.6
75*	August 17	$\left\{ \begin{array}{l} R = 0.040 \\ L = 0.037 \end{array} \right.$	- 8.1				

* Tuberculosis found.

¹ The left suprarenal wholly involved in a tubercle nodule weighing nearly 1.0 gr.² Healthy.³ A hybrid from a family cross.

TABLE 4

Weight, length and width of testes of young Ring dove (specific) hybrids—killed

NO.	DATE	WEIGHT	PER CENT OF DIFFER- ENCE	LENGTH AND WIDTH	PER CENT OF DIFFER- ENCE
88a	December 4, 1915..	R = 0.035 L = 0.030	-16.7	8.0 x 3.8	
89a	December 4, 1915..	R = 0.007(?) L = 0.005(?)	-40.0	5.1 x 1.6 .6 x 1.4	-10.9
90a	December 4, 1915..	R = 0.004(?) L = 0.005(?)	+25.0	4.1 x 0.9 4.9 x 0.9	+19.5
91a	December 4, 1915..	R = less than 0.005(?) L = 0.005(?)	+?	5.1 x 0.8 5.1 x 1.5	=0.0
92	December 4, 1915..	R = 0.005(?) L = 0.005(?)	= 0.0	4.8 x 1.5 5.3 x 1.4	+10.4
93	December 4, 1915..	R = 0.007(?) L = 0.005(?)	-40.0	4.8 x 1.6 5.3 x 0.9	+10.4
94	December 4, 1915..	R = 0.015 L = 0.010	-50.0	6.6 x 1.9 6.6 x 1.5	=0.0
95*	December 8, 1915..	R = 0.025 L = 0.025	= 0.0	8.3 x 2.2 9.0 x 1.9	+8.4
96	December 8, 1915..	R = 0.037 L = 0.025	-48.0	8.4 x 2.7 7.2 x 2.2	-16.7
97	December 8, 1915..	R = 0.010 L = 0.007	-42.9	5.0 x 1.8 5.0 x 1.5	=0.0
98	December 8, 1915..	R = 0.320 L = 0.280	-14.3		
99	December 8, 1915..	R = 0.007(?) L = 0.005(?)	-40.0	4.4 x 1.7 5.0 x 1.0	+13.6
100	December 8, 1915..	R = 0.025 L = 0.020	-25.0	6.8 x 2.2 6.7 x 2.1	-1.5
101	December 8, 1915..	R = 0.010 L = 0.007	-42.9	6.5 x 1.9 6.5 x 1.5	=0.0
102	December 8, 1915..	R = 0.012 L = 0.012	= 0.0	Summary:	
103	December 8, 1915..	R = 0.035 L = 0.025	-40.0	Left larger in.....	2
104	December 8, 1915..	R = 0.100 L = 0.070	-42.9	Left smaller in....	13
105	December 8, 1915..	R = 0.017 L = 0.015	-13.3	Two equal in.....	3
				Left longer in....	5
				Left shorter in....	3
				Left equal in.....	4

* Tuberculosis found.

is probably responsible for the failure of our weighings to differentiate between the masses of several pairs of the testes. In the 30 pairs 18 right testes were larger, 5 were smaller, 7 were not differentiated by the weighings.

Size of testes in hybrids dead of disease

Seven of the 10 specific hybrids of table 2 had larger right testes; 2 had smaller; 1 had the testes of equal size. Of the two generic hybrids (52, 53) represented in this table, one had a larger and one a smaller right testes. In table 3 are listed 13 specific hybrids; 7 larger right testes, 4 smaller, and 2 equivalents.

TABLE 5

Weight, length and width of testes of young Ring dove (specific) hybrids—killed

NO.	DATE	WEIGHT	PER CENT OF DIFFERENCE	LENGTH AND WIDTH	PER CENT OF DIFFERENCE
106	November 29, 1915	R = too small to weigh		3.2 x 1.5	
		L = 0.005	+ 2.0	4.7 x 1.8	+46.9
107	November 29, 1915	R = 0.007		5.8 x 1.6	
		L = 0.007	= 0.0	5.4 x 2.0	- 7.4
108	November 29, 1915	R = 0.009		5.8 x 1.8	
		L = 0.010	+11.1	6.4 x 1.8	+10.3
109	November 29, 1915	R = 0.020		8.2 x 1.9	
		L = 0.019	- 5.3	8.2 x 1.9	= 0.0
110	November 29, 1915	R = 0.018		7.4 x 2.0	
		L = 0.020	+11.1	7.4 x 2.0	= 0.0
111	November 29, 1915	R = 0.010		5.9 x 2.2	
		L = 0.010	= 0.0	7.4 x 1.6	+25.4
112	November 29, 1915	R = 0.190		17.0 x 4.5	
		L = 0.170	-11.8	15.7 x 4.5	- 8.3
113	December 3, 1915..	R = 0.007		5.7 x 1.5	
		L = 0.005	-40.0	4.9 x 0.9	-16.3
114	December 3, 1915..	R = 0.007		5.5 x 1.4	
		L = 0.005	-40.0	4.2 x 1.0	-30.9
115	December 3, 1915..	R = 0.007		Summary: Left larger in 3 Left smaller in 5 Two equal in 4 Left longer in 3 Left shorter in 4 Two equal in 2	
		L = 0.007	= 0.0		
116	December 3, 1915..	R = 0.005			
		L = 0.005	= 0.0		
117	December 3, 1915..	R = 0.007			
		L = 0.005	-40.0		

TABLE 6
Weights and measurements of testes—birds classified as to kind and disease

NO.	DATE	DISEASE ¹	WEIGHTS	PER CENT OF DIFFERENCE	LENGTH AND WIDTH	PER CENT OF DIFFERENCE
Pure species— <i>Spil. tigrina</i>						
118	January 7	Worms	R = 0.050 L = 0.050	= 0.0	7.8 x 3.4 10.8 x 3.0	+38.5
119	January 9	Worms (juv.)	R = 0.012 L = 0.014	+16.6	5.3 x 2.2 7.0 x 1.8	+32.1
120	January 11	Liver	R = 0.040 L = 0.045	+12.5	7.3 x 3.0 9.0 x 2.8	+23.3
121	January 11	Liver and worms ...	R = 0.055 L = 0.055	= 0.0	8.3 x 3.2 8.3 x 3.0	= 0.0
122	January 13	Worms	R = 0.080 L = 0.080	= 0.0	9.3 x 4.5 10.6 x 4.3	+13.9
123	January 15	Worms liver	R = 0.040 L = 0.045	+12.5	6.5 x 3.0 8.2 x 2.8	+26.2
124	January 18	Intest. and liver ...	R = 0.031 L = 0.027	-14.8	5.1 x 3.4 6.7 x 2.7	+31.4
125	January 25	Intest.	R = 0.030 L = 0.045	+16.6	7.1 x 3.0 8.6 x 3.1	+21.1
126	January 29	Worms (old)	R = 0.030 L = 0.022	-36.4	7.6 x 2.6 7.0 x 2.3	- 8.6
127	March 1	Intest. (juv.)	R = 0.004† L = 0.004—	- ? 0	4.2 x 2.1 4.4 x 1.9	+4.8
128	May 17	Sp. Li. (old)	R = 0.047 L = 0.035	-34.3	8.7 7.2	-20.8
129	January 28	Liver and spleen ...	R = 0.030 L = 0.025	-20.0	6.8 x 3.1 7.2 x 2.4	+ 5.9
130	January 28	Worms	R = 0.027 L = 0.030	+11.1	6.7 x 2.5 8.8 x 2.5	+31.3
Pure species— <i>T. orientalis</i>						
131	March 8	Lu. liver	R = 0.016 L = 0.015	- 6.6		
132	March 23	Cold (juv.)	R = 0.010 L = 0.008	-25.0	5.4 x 1.5 3.7 x 1.7	-45.9
133	March 23	Cold (juv.)	R = 0.010 L = 0.008	-25.0	6.0 x 1.4 4.7 x 1.7	-27.6
134	March 23	Cold (juv.)	R = 0.011 L = 0.009	-22.2	7.0 x 1.4 6.3 x 1.5	-11.1
135	March 25	Intest. (juv.)	R = 0.010 L = 0.007	-42.9	5.8 x 1.9 5.5 x 1.6	- 5.5

¹ Abbreviations of the names of organs to their first two letters, implies that advanced and very evident tuberculosis was found in those organs (lungs, spleen, liver, joints, mesentery, intestine). Where more than one organ was affected the name of the organ (or organs) apparently most affected is written first. When the word is written out it denotes that this organ was abnormal, but not necessarily tubercular; immature birds are designated—(juv.).

TABLE 6—Continued

NO	DATE	DISEASE	WEIGHTS	PER CENT OF DIFFERENCE	LENGTH AND WIDTH	PER CENT OF DIFFERENCE
Pure species— <i>St. risoria</i>						
136	December 4	Sp. li. lu.....	$\left\{ \begin{array}{l} R = 0.030 \\ L = 0.025 \end{array} \right.$	-20.0		
137	January 29	Sp. lu. li.....	$\left\{ \begin{array}{l} R = 0.053 \\ L = 0.031 \end{array} \right.$	-70.9	9.2 x 3.3 10.5 x 1.9	+14.1
138	January 29	Sp. lu. li.....	$\left\{ \begin{array}{l} R = 0.015 \\ L = 0.010 \end{array} \right.$	-50.0	6.0 x 2.3 6.6 x 2.0	+10.0
139	February 4	Intest.....	$\left\{ \begin{array}{l} R = 0.037 \\ L = 0.020 \end{array} \right.$	-85.0	8.4 x 2.9 8.8 x 2.3	+ 4.8
140	March 15	Lu. (?) liver.....	$\left\{ \begin{array}{l} R = 0.550 \\ L = 0.401 \end{array} \right.$	-37.1		
141	May 10	Sp.	$\left\{ \begin{array}{l} R = 0.023 \\ L = 0.019 \end{array} \right.$	21.1	8.0 8.0	- 0.0
142	May 14	Jo., lu. sp.; liver...	$\left\{ \begin{array}{l} R = 0.020 \\ L = 0.017 \end{array} \right.$	-17.6	6.7 6.9	+ 2.9

Miscellaneous—pure species

143	November 17	Canker, li., sp. lu...	$\left\{ \begin{array}{l} R = 0.010 \\ L = 0.007 \end{array} \right.$	-42.9		
144	November 17	Fight and hemorr..	$\left\{ \begin{array}{l} R = 0.595 \\ L = 0.475 \end{array} \right.$	-25.3		
145	November 18	Unknown (juv.)....	$\left\{ \begin{array}{l} R = 0.0038 \\ L = 0.0042 \end{array} \right.$	+10.5		
146	November 20	Cold (?).....	$\left\{ \begin{array}{l} R = 0.165 \\ L = 0.127 \end{array} \right.$	-29.9		
147	December 3	Liver.....	$\left\{ \begin{array}{l} R = 0.015 \\ L = 0.016 \end{array} \right.$	+ 6.7		

Common pigeons

148	January 3	Unknown (juv.)....	$\left\{ \begin{array}{l} R = 0.005(?) \\ L = 0.005(?) \end{array} \right.$		5.4 x 1.5 5.2 x 1.3	- 3.8
149	January 17	Unknown (juv.)....	$\left\{ \begin{array}{l} R = 0.009(?) \\ L = 0.010(?) \end{array} \right.$	+11.1	5.8 x 1.5 6.5 x 1.7	+12.1
150	February 15	Li. pleura.....	$\left\{ \begin{array}{l} R = 0.004? \\ L = 0.003? \end{array} \right.$	-33.3?	about 6.0 mm. long about 5.3 mm. long	+13.2
151	April 13	Hemorrhage, liver..	$\left\{ \begin{array}{l} R = 0.014 \\ L = 0.010 \end{array} \right.$	-40.0	6.4 6.4	= 0.0
152	April 17	Appar. healthy.....	$\left\{ \begin{array}{l} R = 1.340 \\ L = 1.315 \end{array} \right.$	- 1.1	20.2 x 12.1 23.9 x 10.7	+18.3
153	May 4	Weakling (juv.)....	$\left\{ \begin{array}{l} R = 0.006 \\ L = 0.004 \end{array} \right.$	-50.0	5.2 5.2	= 0.0

TABLE 6—Continued

NO.	DATE	DISEASE	WEIGHTS	PER CENT OF DIFF- ERENCE	LENGTH AND WIDTH	PER CENT OF DIFFER- ENCE
Hybrids—from crosses of species						
154	January 1	Cold? (juv.).....	R = 0.000 L = 0.000		4.2 x 0.9 5.2 x 1.2	+23.8
155	January 4	Sp. liver.....	R = 0.014 L = 0.017	+21.4	6.3 x 2.1 8.6 x 2.5	+36.5
156	January 8	(Juv.).....	R = 0.000 L = 0.000		3.8 x 1.5 5.7 x 1.2	+50.0
157	January 15	Lu.....	R = 0.037 L = 0.035	- 5.7	8.7 x 2.7 8.4 x 2.8	- 3.6
158	December 3	Li. (juv.).....	R = 0.003 L = 0.003	= 0.0		
159	January 15	Sp. jo.....	R = 0.027 L = 0.020	-35.0		
160	January 27	Healthy dwarf; killed.....	R = 0.010(?) L = 0.013(?)	+30.0?		
161	February 18	Sp. lu.....	R = 0.015 L = 0.015	= 0.0		
162	March 6	Sp. li., etc.....	R = 0.028 L = 0.025	-12.0		
163	January 22	Li., sp.....	R = 0.007 L = 0.007	= 0.0	5.0 x 2.1 5.6 x 2.0	+12.0
164	January 24	Lu., liver, spleen...	R = 0.050 L = 0.050	= 0.0	9.7 x 2.9 10.4 x 2.7	+ 7.2
165	January 27	Liver, lu. (?).....	R = 0.052 L = 0.050	- 4.0	9.0 x 3.7 11.4 x 2.9	+26.6
166	February 13	Lu., liver, spleen (juv.).....	R = 0.002? L = 0.003+	+50.0	5.7 5.9	+ 3.5
167	March 17	Sp., lu. liver.....	R = 0.022 L = 0.021	- 4.7		
168	March 18	Sp. li., lu.....	R = 0.025 L = 0.025	= 0.0	7.2 7.6	+ 5.6
169	March 26	Intest. (?) (juv.)...	R = 0.002 L = 0.002	= 0.0	4.4 3.9	-12.8
170	March 28	Sp. me. lungs.....	R = 0.048 L = 0.042	-14.3	9.9 x 3.7 10.1 x 2.3	+ 2.0
171	March 29	Li. sp., me.....	R = 0.031 L = 0.035	+12.9	8.7 x 2.9 12.2 x 2.2	+40.2
172	April 12	Cold, lungs.....	R = 1.015 L = 0.888	-14.3	22.6 x 9.0 23.5 x 8.2	+ 3.9
173	April 20	(Juv.).....	R = 0.002(?) L = 0.002(?)	= 0.0	5.2 5.4	+ 3.8

TABLE 6 -Continued

NO.	DATE	DISEASE	WEIGHTS	PER CENT OF DIFF- ERENCE	LENGTH AND WIDTH	PER CENT OF DIFFER- ENCE
Hybrids—from crosses of species (con.)						
174	April 23	Liver (intest. ?).....	$\left\{ \begin{array}{l} R = 0.054 \\ L = 0.044 \end{array} \right.$	-22.7	10.8 10.6	- 1.9
175	May 6	Sp. li. pe.....	$\left\{ \begin{array}{l} R = 0.034 \\ L = 0.032 \end{array} \right.$	- 6.3	8.2 8.2	= 0.0
176	May 6	Sp.....	$\left\{ \begin{array}{l} R = 0.020 \\ L = 0.022 \end{array} \right.$	+10.0	7.6 8.0	+ 5.3
177	May 10	Li. spleen.....	$\left\{ \begin{array}{l} R = 0.042 \\ L = 0.036 \end{array} \right.$	-16.7	8.3 8.7	+ 4.8
178	May 11	Sp. li.....	$\left\{ \begin{array}{l} R = 0.021 \\ L = 0.018 \end{array} \right.$	-16.7	7.2 6.6	- 9.1
179	May 16	Healthy (juv.).....	$\left\{ \begin{array}{l} R = 0.125 \\ L = 1.25 \end{array} \right.$	= 0.0	11.6 x 4.5 13.2 x 4.3	+13.8
Hybrids—from crosses of genera						
180	December 4	Sp., li.....	$\left\{ \begin{array}{l} R = 0.017 \\ L = 0.020 \end{array} \right.$	+17.6	7.0 x 2.0 6.3 x 2.5	-11.1
181	December 24	Liver, pericard.....	$\left\{ \begin{array}{l} R = 0.017 \\ L = 0.020 \end{array} \right.$	+17.6		
182	January 8	Cold (?).....	$\left\{ \begin{array}{l} R = 0.005 \\ L = 0.004 \end{array} \right.$	-25.0	3.2 x 1.8 3.0 x 1.5	- 6.6
183	January 12	Abdom. wall.....	$\left\{ \begin{array}{l} R = 0.009 \\ L = 0.012 \end{array} \right.$	+33.3	7.3 x 1.8 5.7 x 1.9	-28.1
184	February 8	Intest.....	$\left\{ \begin{array}{l} R = 0.005? \\ L = 0.004? \end{array} \right.$	-25.0	about 4.5 about 4.5	= 0.0
185	February 23	Intest. (?).....	$\left\{ \begin{array}{l} R = 0.045 \\ L = 0.051 \end{array} \right.$	+12.3	7.3 7.1	- 0.3
186	March 7	(Cold?) lungs.....	$\left\{ \begin{array}{l} R = 0.007 \\ L = 0.005 \end{array} \right.$	-40.0	{ both testes an- gular-globular, like hemip seed	
187	March 13	Li., sp., lu. (?).....	$\left\{ \begin{array}{l} R = 0.014 \\ L = 0.019 \end{array} \right.$	+35.7	7.0 9.0	+28.5
188	March 29	(Cause?).....	$\left\{ \begin{array}{l} R = 0.006 \\ L = 0.007 \end{array} \right.$	+16.7	4.6 x 1.9 5.3 x 2.0	+15.2
189	April 12	Worms, fighting....	$\left\{ \begin{array}{l} R = 0.256 \\ L = 0.212 \end{array} \right.$	-20.8	15.7 x 5.6 15.4 x 5.1	- 1.9
190	May 15	Lu.....	$\left\{ \begin{array}{l} R = 0.047 \\ L = 0.043 \end{array} \right.$	- 9.3	8.8 x 8.0 x	-10.0

Six generic hybrids here all show larger right testes. Of 24 birds listed in table 6, larger rights were found in 11, smaller in 5, equivalents in 8 cases. In table 6, 11 generic hybrids are listed; the right testis is larger in 5, smaller in 6.

A summary representation of the weight relations of the testes of birds belonging to the several preceding groups is given in table 7.

Relative lengths of the two testes

The length of 78 pairs of testes was ascertained. These were obtained from the testes of birds belonging to all of the groups discussed in the previous section of this paper. The reader is referred to the summary on 'length relations' given in table 7 for a first view of the result. Although a high proportion (126 to 39 for all groups) of the right testes are heavier, a reversal of proportions (24 to 42) is found for the absolute lengths of the two testes. In five of the seven groups of table 7 the left testis is absolutely longer. In one of the two exceptional groups—

TABLE 7
Summary of the preceding data

CLASS	STATE	WEIGHT RELATIONS ¹			LENGTH RELATIONS		
		L +	L =	L -	L +	L =	L -
Pure species.....	Healthy...	0	0	5	0	0	3
	Diseased...	9 ²	7 ²	31	14	2	2
Common pigeons.....	Healthy...	5	0	27	1	0	0
	Diseased...	2	0	8	2	2	1
Specific hybrids.....	Healthy...	6	7	19	8	6	7
	Diseased...	10	11	24	15	1	4
Generic hybrids.....	Diseased...	7	0	12	2	1	6
Total healthy.....		11	7	51	9	6	10
Total diseased.....		28	18	74	33	6	14
Grand total.....		39	24	126	42	12	24

¹ The cases of larger left testis, are grouped under L+; those of equal size under L=; those with a smaller left testis under L-.

² Five of the (9), and 3 of the (6), are from 14 Sp. tigrina—all dead at less than 9 months old.

'healthy pure species'—it is possible that the smaller length of the left testis is connected with the nearest approximation to uniformity of smaller size in this group. In the second group—'diseased generic hybrids'—the testes show the greatest departure in their weight relations from what is elsewhere the rule.

An examination of the detailed measurements and percentage weight differences given in tables 4, 5 and 6 is even more convincing than the summary of table 7 on the point that the two testes definitely tend to assume two different shapes—the left to be thinner and more elongate, the right to be shorter and thicker. This difference in form is perhaps not without interest since the only persistent gonad in the female—that of the left side—is characteristically 'thin' and 'long.' The testis that develops on this side is similarly characterized as compared with its mate of the right side.

Pure species and hybrids and the relative size of the two testes

Table 7 facilitates an expression of a relation which seems to obtain between degree of hybridization on the one hand, and the number of departures from the usual situation—a larger right and a smaller left testis—on the other. There are good reasons for believing that common pigeons—mongrels of many breeds probably not even descended from a single good species—may rightly occupy a place in this classification intermediate to specific hybrids and pure species. There is no question that the generic hybrids are more separated from the pure species than are the specific hybrids. Now, the number of gonad size relations, that depart from the rule, arrange themselves in all of the seven groups of the table, precisely in this order of departure from purity of species. That is, the greatest proportion of larger left testes is found in the group most widely separated from a pure species (generic hybrids); and the two intermediate stages of crossing (specific hybrids and common pigeons) show each its appropriately smaller intermediate number of larger left testes.

The influence of disease on the actual and relative size of the two testes

The detailed data of the several tables and the summary of table 7 demonstrate that diseased birds furnish a higher proportion of larger left testes—violations of the more general rule—than do healthy birds. Further, the testes of pigeons suffer great reduction in several, or most, forms of disease. Now it probably happens that because of the great reduction in size of the testes in disease that this of itself has rendered the results of a few of the weighings of the smallest glands less certain.¹ An examination of the detailed data will, however, leave no doubt that disease is a very real and important reason for the observed differences.

It has been found well to designate the cause of death in most of the tables. Advanced tuberculosis is easily, and with much certainty, diagnosed in pigeons. It is the most common cause of death among the birds of our collection. All deaths from this cause are so designated in the tables. A careful comparison of the size of the testes of birds dead of tuberculosis as compared with those dying of other or unknown causes will show that the gonads of the male suffer greatest reduction under this disease. This is certainly not true for the female gonad; a point upon which data are still being collected. In this connection Hatai's ('15) observation of the effects of exercise on the size of the ovary and testis of the rat are of interest. Hatai found a like qualitative response—an increase—in both; but quantitatively the response was quite different; the testes increased only 12.33 per cent while the increase in the ovaries was 84.33 per cent.

The several sections of table 6 show that in pigeons the spleen and liver are more often affected by tuberculosis than are other organs. It is the spleen too that suffers greatest hypertrophy and most complete transformation under the disease. This is true of both sexes.

¹ Because of imperfect or unclean separation of the gland from body wall, and drying during weight, the greatest care will not always obtain perfect weights of the smallest of these glands.

Size relations of the testes in the common fowl

In table 8 are given the few data we have been able to obtain on the Jungle fowl, common fowl, and the duck. Since these were—with two exceptions—healthy fowls even the few data indicate a situation different from that found in pure species of pigeons. Whether these data are really representative of these forms, cannot now be determined. Whether hybridization (or mongrelization) of these forms is responsible for the apparent predominance of the reverse of the situation found in pigeons is doubtful. They like the sparrows may normally possess a larger left and a smaller right testis.

TABLE 8
Weights of testes of Jungle Fowl and common fowl

NO.	DATE	WEIGHT	PER CENT OF DIFF- ERENCE	NO.	DATE	WEIGHT	PER CENT OF DIFF- ERENCE
Jungle fowl				Common fowl			
1	July 5.....	R = 3.203 L = 4.217	+31.7	1	July 16.....	R = 3.734 L = 4.219	+12.9
2	July 5.....	R = 4.700 L = 5.700	+21.3	2	July 16.....	R = 5.880 L = 7.000	+19.0
3	July 8.....	R = 4.635 L = 4.280	- 8.4	3	July 20.....	R = 6.540 L = 5.610	-16.6
4	July 10.....	R = 6.305 L = 6.270	- 0.6	4	July 22.....	R = 11.660 L = 12.815	+ 9.9
5	July 12.....	R = 4.500 L = 4.900	+ 8.9	5	August 26....	R = 8.820 L = 7.545	-16.9
6	July 16 (inj. ¹)	R = 3.960 L = 5.105	+28.9	6	April 21.....	R = 11.100 L = 10.350	- 7.2
7	July 22 (inj. ¹)	R = 1.665 L = 1.820	+ 9.3	7	December 10 (roup).....	R = 0.235 L = 0.200	-17.5
				8	December 21 (roup).....	R = 0.590 L = 0.635	+ 7.6
Wild Duck							
1	(y'g) Novem- ber 29.....	R = 0.023 L = 0.027	+17.4	2	December 28 (starved).. R = 8.3 x 3.2	R = 0.040 L = 0.040 L = 8.6 x 2.5	0.0

¹ These cocks had been given a few injections of ovarian extract during the week preceding the days of killing and autopsy.

SUMMARY

The prevalence of atrophy of the right ovary in birds; the demonstrated differences in number of primordial germ cells in the two glands of the fowl; and the unequal—and opposite—size relations of the two adult gonads of the male, constitute a body of puzzling facts whose elucidation should contribute largely to our knowledge of the nature and basis of sexual difference.

The right testis of the pigeon is normally larger than the left.

In hybrid pigeons there are more exceptions to the normal size-relations of the two testes than in pure species. The number of the exceptions seems to increase with the degree of hybridization (width of the cross); there being fewer in specific hybrids than in generic hybrids.

The testes of pigeons suffer great reduction in size in disease—particularly in tuberculosis. It is probable that the right gland suffers greater reduction than the left. The left (persistent) gonad of the female does not suffer a similar reduction in tuberculosis. Season is plainly not the cause of the differences and reductions noted in pigeons.

The two testes of the pigeon are characteristically different in their dimensions. The left (like the left ovary) is thinner and more elongate. The right (represented in the female by atrophied ovary) is shorter and thicker.

In poultry the few data at hand fail to indicate a constant or decided predominance of size in either gland.

Cold Spring Harbor, L. I., N. Y. June, 1916

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HYGIENIC CAGES FOR RATS AND MICE

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TWO FIGURES

The following is a brief account of cages recently constructed for the Department of Anatomy of the University of California for housing colonies of rats and mice.

In planning these cages the desirability was kept in mind of so designing them that not only might the animals be cared for conveniently, but the cages be easily cleaned and completely sterilized and the spread of infection prevented. Accordingly they were made entirely of metal: the sides and front of galvanized iron, the former preventing the direct passage of infection from cage to cage; and the lid, top, back, and floor of hardware cloth of $\frac{1}{4}$ -inch mesh bound with strips of galvanized iron. They can be taken apart, packed in a small space for boiling, and reassembled quickly without the use of any screws or bolts. All parts are interchangeable. The inside dimensions are: floor, $9\frac{1}{2}$ by $14\frac{1}{2}$ inches; height 11 inches; front $2\frac{3}{4}$ inches high.

They are arranged in groups of 20 (4 rows of 5 each, fig. 1). Each group is supported by a rack made of iron pipe, and 4 pairs of angle irons on which the 4 rows of cages are hung. Below each row is placed a shallow, removable, galvanized iron pan intended to be filled with sawdust for receiving refuse falling through the bottoms of the cages. The racks measure $6\frac{1}{2}$ feet in height, $17\frac{1}{2}$ inches in depth, and $4\frac{1}{2}$ feet in width. There is a space of $9\frac{1}{2}$ inches between the lowest pan and the floor, and 3 inches between the floors of the cages and pans. If desired the racks can be continued upward to carry one or more additional rows.

Most of the details of construction can be seen in figure 2 which shows some of the cages taken down. It will be observed that the sides are suspended and in turn furnish support for the rest of the cage except the top. The sides are put into place by slipping the flanges on the upper edges into grooves (*gr* fig. 2) formed by bending-under the edges of strips of galvanized iron (*st*). A projection (*pr*) prevents sliding the sides in too far. The ends of the strips forming the grooves are bent up and over the angle irons and are permanently fastened by means of bolts. These strips also have soldered to their upper sides grooves (*lg*) opening laterally formed by strips of metal bent in the form of a narrow trough. Into the latter slide the tops to which the lids are hinged by two rings. The backs when in place





rest on the flanges on the lower edges of the sides and against the front faces of the back flanges. The upper ends of the backs are held firmly because they pass behind the rear angle irons; at the lower ends pins (p) fit into holes in the bottom flanges (the pins can also be seen on the under side of the upper row of cages). The binding on the lower edge of each back is turned forward at a right angle and together with the flanges on the lower edges of the sides serves to support the floor. The latter are kept in place by two pins (p') which fit into corresponding holes (h) in the binding. The front is made of one piece of metal. One may be seen endwise resting on the edge of a tray. The ends are bent somewhat in the form of a letter S to form troughs which fit over the flanges on the front edges of the sides.

In assembling the cages the sides are first put into place, then the backs, floors, front, and top (with lid). It will be seen that the floors may be changed without disturbing the rest of the cage, or by removing simply the front. A number of extra bottoms makes it possible to clean one set and have them ready to substitute for soiled ones every week. The other parts of the cages need cleaning only at longer intervals.

For the cages used for rats, floors of $\frac{1}{2}$ inch mesh are provided.

It has been found in actual breeding that 4 and even 6 adult rats can be kept in one cage, and as many as 10 or 12 young rats raised to breeding age in single cages.

The construction of this equipment was worked out by Prof. H. M. Evans and the writer with assistance from Mr. H. B. Foster, the University Engineer.

THE GOLGI APPARATUS

PERSONAL OBSERVATIONS AND A REVIEW OF THE LITERATURE

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TWENTY-TWO FIGURES

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I. INTRODUCTION

It is rather extraordinary that there should be within every cell a structure as conspicuous as the nucleus, and sometimes surpassing it in size, the meaning of which is utterly obscure. One at least of the functions of the nucleus—its rôle in heredity—is known to us. We have fairly definite ideas as to the rôle of the centrosomes, and theories aplenty as to the part played by mitochondrial structures, and other types of granules. But as regards the structure to which Golgi has given the name 'Apparato reticolare interno,' we have learned only its appearance, its distribution in different types of cells, and its behavior dur-

ing cell division. One of the most recent papers on the subject—that of Kolster (102)—ends with the statement “These structures undoubtedly have a special significance, but we are ignorant of it.”

The credit for the discovery of this intracellular organelle—if such it be—undoubtedly belongs to Golgi, and a large part of the work, including the working out of a fairly easy and satisfactory technique for its demonstration—has been done by Golgi himself, and by his pupils and co-workers, Veratti, Perroncito, Pensa, Negri, Gemelli, Brugnattelli and others. A number of papers dealing with the same structures have been published by Ramon-y-Cajal, who, indeed claims priority for their discovery over Golgi, and by his pupils, Sanchez, Fananas, Tello and others. Nussbaum, in Prague, has inspired a series of papers by his pupils (Weigl, Polyeszynski, Bialschowska and Kulikowska) dealing chiefly with the appearance of the Golgi apparatus in the ganglion cells of invertebrates. Important papers have been published by v. Bergen, Deineke and by Kopsch, who discovered a new and very simple method for demonstrating the apparatus. The best and most exhaustive general review on the subject is that of Duesberg (48), before the XXVIII Meeting of the Anatomische Gesellschaft at Innsbruck in 1914, and Cajal (31) in his most recent publication ('15) which was not available at the time this study was begun, has contributed a most interesting critical survey of the entire field, and added many new observations.

A whole chapter—largely controversial—is that contributed by Holmgren, whose views and their bearing I shall take up later. In this country only Bensley and Cowdry have made contributions to the subject.

In reviewing the literature of the subject I found that but few workers, with the exception of Cajal and his pupils, had attempted to study the behavior of the Golgi apparatus under experimental conditions. I planned, therefore, to follow the modifications of the structure in the epithelial cells of the rat kidney, which might be produced by autolytic changes, secretory phases and toxic agents. The choice of material was unfortunate. The Golgi apparatus of the cells of the renal tubules

proved to be so atypical and variable in form that it was difficult to draw inferences from variations seen under experimental conditions. One was further handicapped by the difficulties and capriciousness of the impregnation method, as applied to this organ.

In attempting to control the technique many other tissues were studied, and insofar as the observations made differ from those of previous workers, they are given below.

Although I was unsuccessful in the main purpose of my study, it seemed that it might be useful at this time to collate the widely scattered and rather inaccessible literature, and to record my personal observations, insofar as they supplement or are at variance with those of other workers in this field.

II. NOMENCLATURE

Golgi (61), in his original communication before the Med. Chir. Society of Pavia in 1898, suggested the term 'Apparato reticolare interno,' and this term has naturally been adopted by all the Italian workers. Kopsch (103) proposed the term 'Binnennetz' as the German equivalent, but both of these designations are open to the objection that the structures do not appear in all types of cells, nor under all conditions, as a closed net. Ballowitz (4) in 1899 described a basket-like structure about the centrosomes of the cells of Descemet's membrane, and suggested for it the name 'Centrophormia.' Later he recognized the homology of the 'Centrophormia' with the Golgi apparatus, and the term has not come into common use. The 'Nebenkern' of Platner (145) and la Valette St. George (106), and the 'Zentralkapsel' of Heidenhain (68) in the sperm cells have been considered by some as related to or identical with the structures demonstrated by the Golgi technique. The terms, however, are not sufficiently inclusive to apply to the structures described by Golgi. Ramon-y-Cajal and his school who agreed with Holmgren in regarding the apparatus as canalicular in nature—referred to it in their earlier publications as the Holmgren-Golgi apparatus. In his latest review, however, Cajal (31) recognizes the very doubtful identity of many of the structures described by Holm-

gren with those brought out by the Silver methods, and therefore refers to them more justly as the Golgi apparatus. Many of the German workers speak of the Golgi-Kopsch net or apparatus.

Holmgren (90)ⁱ, who believes in the identity of the canaliculi described by him, with the structures put in evidence by the silver impregnation methods, uses the term 'Trophospongium.' Cowdry (42) and Bensley (14) speak of 'canalicular apparatus.'

None of these terms appear to be entirely satisfactory. I shall, therefore, refer to the structures simply as the Golgi apparatus.

III. TECHNIQUE

The earliest studies of Golgi were made with a modification of his well-known silver chromate method. This gave capricious and inconstant results. Veratti introduced a modification, the essential feature of which was a fixation in osmium platinic chlorid mixture. Kopsch (103) in 1902 showed that prolonged immersion in 2 per cent osmic acid would demonstrate structures identical with those described by Golgi.

The two methods now most commonly used are those of Golgi (66) and of Cajal (30), and, for the convenience of those to whom the original articles are not accessible, they are given here:

The Golgi method is as follows:

- I. *Fixation:* Formalin (20%)..... 30 cc.
Saturated solution arsenious acid (1%)..... 30 cc.
Alcohol (97%)..... 30 cc.
6 to 24 hours.
- II. *Silver nitrate* 1%..... 1 hour to several days
- III. *Development:* Hydroquinone..... 20 gm. }
Sodium Sulphite..... 1 gm. } 2 to 3 hours
Formalin..... 20 cc. }
Distilled water ad 1000 cc.
Wash in distilled water, dehydrate rapidly and embed
in paraffin or celloidin.
- IV. *Toning:*
Solution 'A' Sodium hyposulphite..... 30 gm.
Ammonium Sulphocyanate..... 30 gm.
Distilled water..... 1000 cc.
Solution 'B' Gold chloride..... 1%
Use equal parts of 'A' and 'B'. Tone to grey tone.

Veratti has devised the following procedure for ridding the preparation of silver precipitate after toning:

- 'A' Repeated washing in distilled water.
- 'B' Rapid passage through following solutions:
 - (1) Potassium permanganate—0.5 gm.
 - Sulphuric acid—1.0 cc.
 - Distilled water—1000 cc.
 - (2) Oxalic acid—1%

Wash in distilled water. Counterstain with alum carmine.

The latest Cajal method differs from the Golgi chiefly in the use of uranium nitrate in place of the arsenious acid in fixation. It is given as follows:

- I. *Fixation*: Uranium nitrate—1 gm.
- Formol—15 cc. } 9 to 11 hours
- Distilled water—100 cc.
- II. Wash quickly
- III. Silver nitrate—1.5%—30 to 40 hours
- IV. Wash quickly
- V. Reduce in
 - Hydroquinone—2 gm.
 - Formol—6 gm.
 - Distilled water—100 cc.
- Add anhydrous sodium sulphite—0.15–0.25 gm. so that solution has a yellow color.
- VI. Dehydrate and embed in paraffin.
- VII. *Toning*:
 - 'A' Sodium hyposulphite—3 gm.
 - Ammonium sulphocyanate—3 gm.
 - Distilled water—100 cc.
 - 'B' Gold chloride—1%
 - Use equal parts.

The addition of 30 cc. of ethyl or methyl alcohol to the fixative is recommended by Cajal (15), as advantageous in the case of nervous tissue.

As counterstain I have found a dilute Giemsa solution to give the clearest pictures. A 1 per cent methyl-green solution may also be used, and it has been found possible to combine also the Altmann mitochondrial stain, as modified by Bensley.¹⁵

The removal of the silver precipitate with permanganate and oxalic acid must be very carefully controlled, as it is easy to bring about a complete decolorization of the Golgi apparatus as well.

Cajal and others have obtained the most constant results in the tissues of young animals. Because of the rapid occurrence of autolytic changes little confidence can be placed in the results obtained with tissues from human autopsies.

Both the Golgi and Cajal methods are exceedingly capricious; the impregnation is rarely uniform throughout the entire block.

The most delicate and important step in these photographic processes, according to several workers, is the initial time of fixation. Each type of cell has its optimum time of fixation, which must be determined experimentally. In many cells, however, as in the lymphocytes, spermatie cells, glomeruli of the kidney, this appears to vary within wide limits. That, at least, has been my experience, and I have obtained identical pictures with fixation varying from 2 to 12 hours.

The technique most recently advocated by Holmgren for demonstrating his Trophospongium is a fixation in trichloroacetic acid (6 per cent) and staining in a freshly prepared resorcin-fuchsin solution. The 'canals' take a purplish black color. Holmgren also gives methods which show the canals as colorless structures upon a stained background.

IV. THE OCCURRENCE OF THE GOLGI APPARATUS IN VARIOUS TYPES OF TISSUE CELLS

1. *Nervous tissue*

The first clear description of the structures is that of Golgi in 1898 (61, 62), in the spinal ganglion cells of *Strix flammea* (Barn-owl); in the same year, he made similar observations upon the spinal ganglion cells of Mammalia; Veratti in 1898 found the same sort of structure in sympathetic ganglion cells. Since these early papers, the Golgi apparatus has been found to be present in many other types of nerve cells—the anterior horn cells (Golgi (66), Cajal (31)), the pyramidal cells of the cortex (Golgi (65), Legendre (107), Collin and Lucien (37), Soukhanoff (167), Cajal (31)), the Purkinje cells and other nerve cells of the cerebellar cortex (Golgi (66), Cajal (31)), of the olfactory lobe (Cajal (28)), the ganglion cells of insects

(Bialkowska and Kulikowska (19)), of the leech and earthworm (Bialkowska and Kulikowska), Crustacea (Jawarowsky (98), Monti (127), Polenzynsky (146), of cephalopods (Weigl (180)).

The apparatus reaches its greatest complexity and size in the spinal ganglion cells of vertebrates, and these have, therefore, been a favorite object of study. In adult vertebrates there is shown by the silver or osmic methods, a definite network of solid, tortuous varicose fibrils, which vary in thickness with different species. This network may completely or partially surround the nucleus and may be in contact with it in places. Where the threads cross or interlace, there are often nodular varicosities. In some species there is a sort of lobulation, into three or four partially separated skeins, and individual filaments may be given off from the main mass, and apparently end freely in the cytoplasm. In all cases the peripheral zone of cytoplasm is left free; at no point does the network, or any of its branches reach the surface.

Monti (127), in the ganglion cells of invertebrates (crustacea, arthropods and cephalopods) found a simple apparatus in the form of curved filaments, often bifurcating or anastomosing, but not forming a closed reticulum. V. Bergen (10), working with the Kopsch osmium method, upon the spinal ganglion cells of the hedgehog, cat, rabbit, rat, mouse, and hen, found that not all the cells showed a complete reticulum as described by Golgi, some containing only short filaments, rows of granules or ring forms. Some of the filaments contained a central clear space, and these he interpreted as degeneration forms. This variation in the appearance of the apparatus in different cells in the same preparation v. Bergen interprets as indicating the transitory nature of these structures. He suggests that they are developed from granules, which range themselves into filaments, form more complex networks, and finally undergo central liquefaction with the formation of canaliculi. Other recent workers, however, using the newer methods of Golgi and Cajal have not confirmed v. Bergen's theory, and ascribe the variations to defective impregnation.

Cajal (31), like v. Bergen, notes variation (or 'modalities') in the type of net occurring in ganglion cells of the same order and size. He strongly rejects the idea that these variations are due to irregularities in impregnation, since they may be found in adjoining cells at similar depths from the surface.

The question has arisen as to whether the Golgi apparatus is identical with any of the other known cytoplasmic constituents of the nerve cell—namely, the neurofibrillae, the Nissl substance or the mitochondria. It seems quite certain, in spite of occasional statements to the contrary, that the Golgi net is unrelated to any of these structures. The net is not continued into the cell processes, as are the neurofibrillae, and the fibers of the net are much thicker and more varicose. By combining Kopsch's method with Bensley's aniline-fuchsin toluidin-blue stain, as Cowdry (42) has done, the Golgi net, Nissl bodies and mitochondria may all be stained in the same cells, and their independence of one another made obvious. It seems hardly worth while to go further into this discussion.

2. *Tissue cells other than nerve cells*

a. Epithelial cells. The presence of a Golgi apparatus was first demonstrated in the squamous epithelial cells of *Ammocoetes* (Lamprey eel) by Marengi (120) in 1903, and in *Lumbricus* by Ramon-y-Cajal in the same year. Since then it has been found in the corneal epithelium by Barinetti (6) and by Deinecke (47). The net is present in all layers. In the superficial cells the net becomes looser, and often almost entirely surrounds the nucleus, whereas in the rete mucosum, it lies at the superficial pole of the nucleus, and in the cells near the surface, only granular bodies are found. This change, therefore, accompanies the aging of the cells, and is characteristic not only of corneal epithelium, but also of skin, oesophageal mucosa, the epidermis of the ducks bill (Deinecke, Kolmer (100)). Some of our preparations of the mucosa of the renal pelvis show a similar differentiation.

A Golgi net has been found also in cells of the epidermal appendages and glands—in the lachrymal gland by Ancona (3)

and in the sweat and sebaceous glands by v. Bergen (10), by Bizzozero and Bottisella (21) (who picture a net surrounding the nucleus, and also, incidentally, could not demonstrate it in the epidermal cells), and by Tello (172).

Since the first paper of Ballowitz (4) in 1898, a net has been found in the single layered epithelial cells of Descemet's membrane by Totsuka (174), Zawarzin (184), and by Deinecke (47). The net in these cells is of special interest because it very clearly lies in relation to the centrosomes, and because it was discovered independently of Golgi's work by Ballowitz. Deinecke in these cells, also, made a careful study of the behaviour of the net during mitosis.

Numerous observations confirm the presence of a Golgi apparatus in the glandular cells of the gastro-intestinal tract. Thus Ramon-y-Cajal ('03) found it in the intestinal epithelium of lumbricus, and of the guinea pig (27); v. Bergen (10) in the chief cells of the fungus region ('04), Golgi (67) in the gastric and intestinal mucosa of frogs, birds and mammals, in the glands of Brunner and of Lieberkuhn ('09); d'Agata (43) in the gastric epithelium of triton ('10) and in the gall-bladder epithelium of the guinea pig (44); Weigl (180) and Kolmer (100) in the gastric and intestinal mucosa of various vertebrates; Kolster (102) in the chief and parietal cells of the fundus, in the pylorus and in the cells of Brunner glands ('13).

Kolster has made several interesting observations on the behaviour of the Golgi apparatus in the gastric cells. He found that when the chief cells were successfully impregnated the parietal cells were not. He also showed that, by using the original Golgi silver chromate method, it was possible to impregnate a system of endocellular excretory canals in the chief cells of the fundus, and that these differed, both in their topography and in their form from the true Golgi apparatus. He noticed also in the pyloric gland cells that the appearance of the net varied with different phases of secretion. In the resting cells the net was quite dense, the meshes small, the form of the whole mass spherical; while in secreting cells, the net was rare-

fied (aufgelockert), elongated and extended to the basal portion of the cell, in close contact with the flattened nucleus.

Cajal (31) also describes in great detail a cycle of changes corresponding to different secretory phases in the goblet cells of the alimentary tract. The Golgi apparatus during the earlier phases undergoes an increase in size, later the argentophile substance becomes dispersed amongst the globules of secretion, and completely disintegrates—not as Kolster (102) believes, becoming merely compressed against the nucleus at the base of the cell.

The inferences which Cajal draws as to the functional significance of these cyclical changes, will be discussed later.

Before having access to Cajal's paper, I had independently observed similar alterations in the mucous glands of the larynx (figs. 1, 2, 3). It seems to be quite clear that the net, which is more distinct and well-formed in cells during the inactive stage, becomes broken up and distributed amongst the globules of mucus in those cells which are actively secreting. In the course of this process, there appears to occur a real quantitative decrease in the amount of the argentophile substance not to be explained merely by its mechanical disruption, and implying some sort of regeneration of the apparatus, after the cell has discharged its secretion and returned to rest.

A Golgi net has been demonstrated by numerous observers in the epithelial cells of various glandular organs, and I shall limit myself merely to giving a list of these. The Golgi net was described in the thyroid by Negri (130) and by Kolster (102); in the adrenal medulla by Pensa (135) and Kolmer (100), and in the cortex by Pilat (144), by Mulon (128) and by Kolmer (100); in the anterior lobe of the hypophysis by Gemelli (60), and in both glandular and nervous portions by Tello (172); in the pancreas by Negri (130), by v. Bergen (10) and by Kolster (102), Kolmer (100) and Cajal (31); in the dog's prostate by v. Bergen (10) and in the hypertrophied human prostate by Verson (179) and by Taddei (171). Von Bergen claims to have been able to recognize the net in unstained scrapings of prostatic epithelium kept alive for a time in the prostatic secretion.

This appears to be the only recorded attempt to observe the Golgi apparatus in the living cell.¹

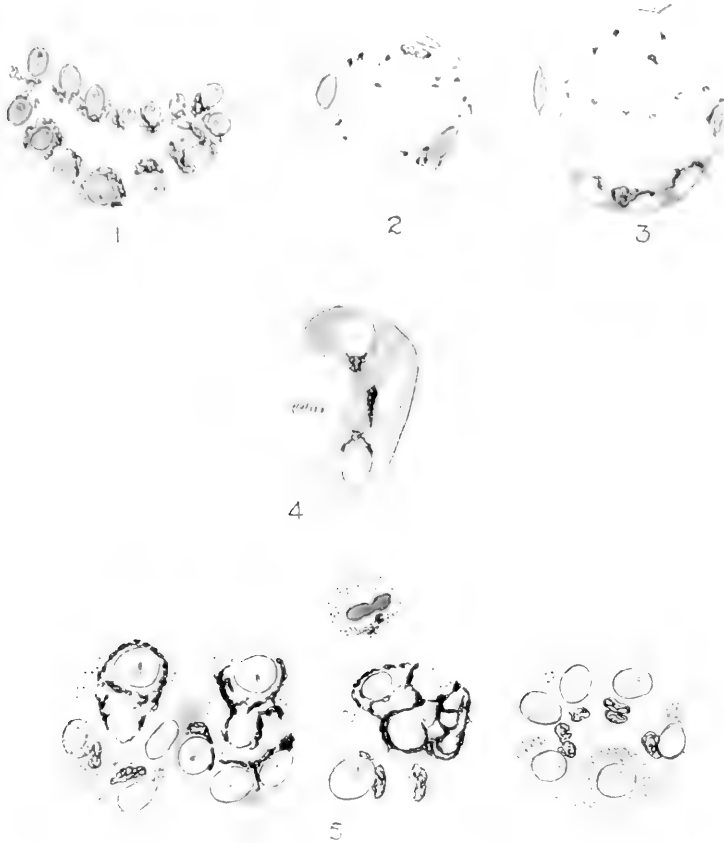


Fig. 1 Tracheal gland, non-secreting. Cajal.

Figs. 2 and 3 Tracheal glands, showing fragmentation of Golgi apparatus during secretion of mucus.

Fig. 4 Small group of thyroid epithelial cells, one in mitosis. Dittokinesis. Cajal.

Fig. 5 Salivary gland of rat. Cajal, anilin-fuchsin-methyl-green. Note relation of Golgi net to Altmann granules.

¹ We also have tried to observe it in growing chick embryo cells in vitro, both by direct light and using dark-field illumination, but without success. Lewis and Lewis (109) likewise report their inability to see structures corresponding to the Binnennetz in living chick embryo cells, nor could they be brought out by prolonged osmic acid fixation.

The net has been found further in the epithelial cells of the epididymis by Negri (130), by Fusari ('08), by Kolster (102) and by Kolmer (100); in the ciliated epithelium of the trachea by Kolster (102); in the choroid plexus by Biondi (20) and in the uterine mucosa and chorionic epithelium by Decio (46) and by Accenti (1).

Negri (130), Kopsch (103), v. Bergen (10), Kolster (102) and Kolmer (100) and Cajal (31), record the presence of a net in the salivary gland epithelium. My preparations show one or two points which I do not find mentioned in their descriptions.

I find in some acini remarkably large, coarse-meshed nets, enveloping the nucleus, joining by stout, varicose filaments with nets in adjacent cells, and not infrequently giving origin to trabeculae which loop about the lumen of the gland. They are thus not confined to a single cell, and anastomose freely one with another.

By counterstaining the Golgi preparations by the anilin-fuchsin-methyl green modification of Altmann's method, one can clearly recognize the independence of the net from the Altmann granules, which are evenly distributed through the entire cell. Indeed, it seems as if these were an inverse relation—that is, those cells in which the granules are poorly marked and absent, show the most conspicuous and clearly defined net, whereas the large cells which are replete with granules, may contain no net at all. Whether this is a constant relation or not, remains to be seen (fig. 5).

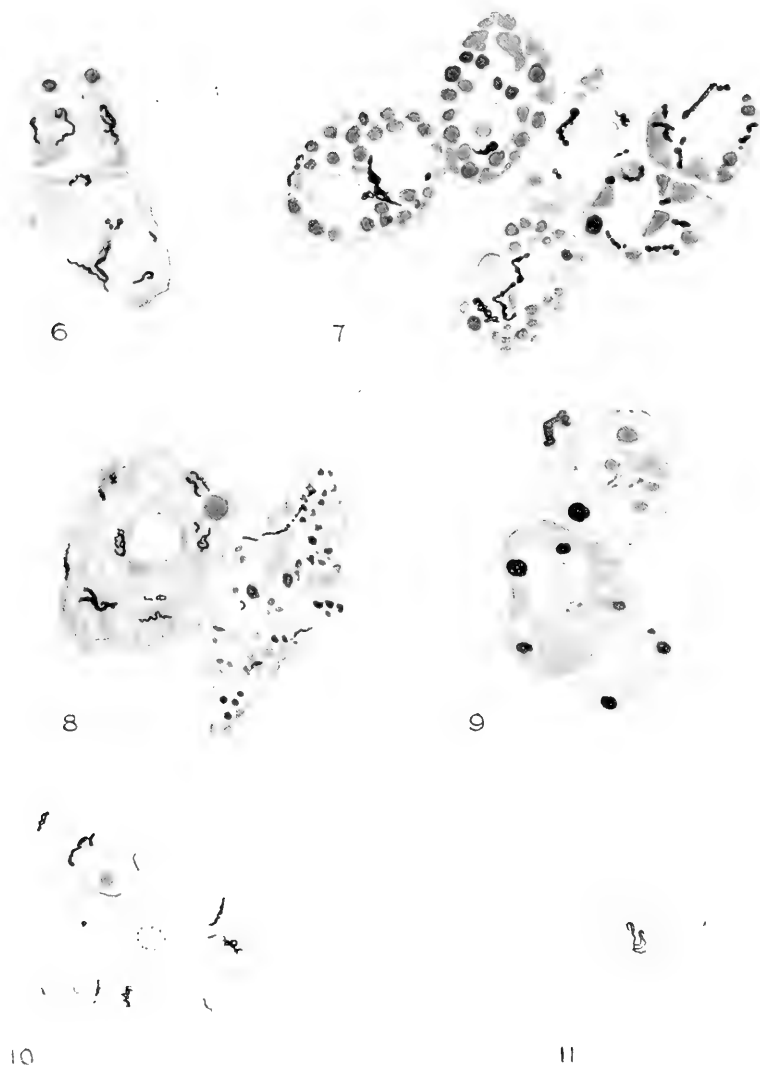
It is surprising that the literature should contain but two references to the presence of a Golgi apparatus in the liver cells. Stropeni (169) is the only one who has succeeded, and he stated that in mammalian livers he obtained only a partial impregnation. With the livers of lower vertebrates, namely frogs and amphibians (Axolotl) he was more fortunate. He found the net to be definitely localized to the portion of cytoplasm between the nucleus and the bile-canaliculi, occasionally sending prolongations into the rest of the cytoplasm; no continuity with the bile canaliculi could ever be observed, nor was there any striking difference in the appearance of the net in fasting or well-fed animals.

Kolmer (100) says that he succeeded but rarely in demonstrating a net in the liver cells. In a new-born cat, the liver cells contained a simple juxta-nuclear net consisting only of several meshes or sometimes of single polygonal nets with one or two long processes. They had no constant relation to the nucleus.

We also have tried repeatedly to find a Golgi apparatus in the liver cells of rats, and have been almost uniformly unsuccessful with the Golgi or Cajal technique. In only one preparation were there found discontinuous curved filaments distributed through the cytoplasm, and bearing little resemblance to the complex reticulum found in other epithelial cells. By prolonged fixation in 2 per cent osmic acid, one may, however, demonstrate very clear-cut intracellular filaments and rows of granules, often curved and occasionally branching, but never uniting to form a definite network (figs. 6, 7, 8, 9, 10, 11). These filaments may lie against the nuclear membrane; in a few instances they appear to join filaments in neighboring cells; in no case do they connect with the bile canaliculi, nor do they appear to reach the surface of the cell. Whether these structures are the homologues of the Golgi apparatus in other cells, I am unable to say with certainty. Their resistance to impregnation by the usual methods implies some chemical variation. They disappear rapidly during autolysis and are absent in cells injured by chloroform poisoning.

Surprisingly few workers also, have concerned themselves with the Golgi apparatus as it appears in the kidney.

Brugnatelli (25), using the Golgi arsenic method has described a net in the cells of the tubuli contorti and of the tubuli recti of the guinea pig, which coincides perfectly with that of other epithelial cells, especially as regards its localization between the nucleus and the lumen of the tubule. In the collecting tubules the apparatus was much more complicated and definite than in the cells of the convoluted tubules, in which it invariably presented itself as small, very simple, almost simulated (i.e., 'accenata'). It seemed, he says, as if the more complex structures here (basal-rods, granules) were harmful to a clear-cut demonstration of the reticular apparatus.



Figs. 6, 7 and 8 Liver cells of rat. Kopsch, 2 per cent osmic acid, 12 days.

Fig. 9 Liver cells of rat, autolysed 3 hours at 37°. Kopsch.

Fig. 10 Liver cells, rat. Cajal.

Fig. 11 Liver cell, rat, vacuolated with localized juxta-nuclear Golgi apparatus. Cajal.

In the glomeruli, the apparatus is reduced to its lowest terms—sometimes appearing as a simple nodule, or as a small figure 8, and always lying adjacent to the nucleus. Brugnattelli gained the impression that the net was restricted to the cells of epithelial origin forming the visceral layer of Bowman's capsule. He is quite wrong in this, as the endothelial cells and the parietal cells of the capsular space also contain an easily demonstrable net (fig. 12).

Barinetti⁶ ('12) describes and pictures a rather complex net in the renal epithelium, and shows its relation to the centrosomes by comparing it with impregnations in which the centrosome is stained by Benda's method. He omits, however, to mention the portion of the renal tubule to which he refers.

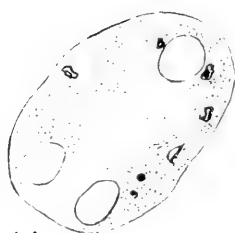
San Giorgi (156) studied the alterations of the Golgi apparatus during experimentally produced nephritis in guinea pigs. He used as toxic agents, uranium nitrate, cantharidin, ricin and diphtheria toxin.

The modifications observed were a splitting up of the filaments or a granular fragmentation without complete loss of the reticular character. Such modifications were most clearly observed in the tubuli recti of the medulla, in which the net, as Brugnattelli showed, is normally more voluminous and complete than in the cells of the convoluted tubules. Close examination showed relation between alterations of the cells as a whole and of the Golgi apparatus. The fragmentation of the net may be marked in some cells of the tubules, whereas others may contain a normal net.

One may criticize San Giorgi's work because of the fact that none of the poisons used produce obvious changes in the cells of the tubuli recti.

Kolmer (100) briefly records the presence of a net in various elements of the kidney, but gives no detailed description.

We have made numerous preparations of rats' kidneys, both of normal animals and of animals in which a uranium nitrate nephritis had been produced. We have varied the time of fixation from two hours (as recommended by Brugnattelli) to twelve hours, without obtaining striking differences. We have also



studied, though incompletely, the effect of autolysis, and found that changes were discernable only after an hour at 37° when the kidney was removed from the body immediately after death. A kidney removed from an animal one hour after death, showed about the same type of structure as the freshly fixed tissue. The apparatus, therefore seems to be somewhat more resistant to autolytic change than the mitochondria, which after half an hour at 37° were broken up into coarse droplets.

As regards the appearance of the Golgi apparatus in the cells of the convoluted tubules, my preparations do not coincide at all with those of Brugnattelli (25) or San Giorgi (156). The argentophile structures in these cells take on the most bizarre and varying forms. One finds smaller and larger droplets or granules, rings or signet forms often ending in a delicate filament, curved threads, uniform in calibre, or with nodular thickenings and varicosities; and larger, more complicated skeins approaching the reticulum described in other types of epithelial cells (figs. 13, 14, 15, 16). The location of these structures with respect to the nucleus is as variable as their form. Their most frequent site is perhaps at either side of the nucleus, sometimes in contact with it; the filaments in general tend to run at right angles to the basement membrane. Sometimes one finds a cluster of granules and short filaments in the supra-nuclear zone, very rarely between the nucleus and the basement membrane. The appearance varies also from one tubule to another in the same preparation; some tubules may show predominantly irregular twisted threads and skeins, others only granulae of uniform or varying size. The appearances are so bewildering in their variety that it is difficult to draw any conclusions, and we are still experimenting with the technique in the hope of getting more constant pictures. We have about decided, however, that the Golgi apparatus in the convoluted tubules is not the

Fig. 12 Rat kidney. Glomerulus. Cajal.

Fig. 13 Rat kidney. Proximal convoluted tubule. Cajal.

Figs. 14, 15 and 16 Rat kidney removed one hour after death. Convoluted tubules. Golgi.

Fig. 17 Rat kidney. Large Henle tubule. Impregnation of basal filaments. Golgi.

clear-cut definite structure which Brugnattelli depicts, but is normally fragmentary and dispersed. We have not been able to prove that these variations in form are correlated with different phases of secretion.

In the Henle tubules, the net is obscured by a very constant impregnation of the Stäbchen. Sometimes, however, we can distinguish a small, very dense, supra-nuclear network, and occasionally filaments are continued along the lateral aspects of the nuclear membrane (fig. 17).

In the cells of the collecting tubules also, a net has been frequently seen, although in the rat's kidney it is a looser and less complex structure than that described by the Italian workers.

In the glomerular cells, both epithelial and endothelial, the small juxta-nuclear net is constantly found, and very sharply defined and striking (fig. 12).

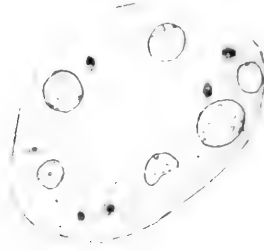
In the uranium nitrate kidney I have made only a few observations which seem worth mentioning at this time.

In the first place, the more complicated filaments and skeins, usually evident in the normal tubules, tend to disappear entirely in the injured kidney. Even those cells, which in sections counterstained with Giemsa, show little or no obvious damage, rarely show structures other than granules or irregular black or grey-staining clumps. In the totally necrotic and desquamated cells, one often finds a single coarse black clump possibly representing the remains of the argentophile structure.

In many of the injured cells I find oval or circular greyish bodies of varying size, many of which contain one or two eccentrically placed black granules (fig. 19). Now starting with these, one can trace transitions both to small solid black granules and to large droplets which have entirely lost their affinity for the silver-stain, taking the eosin of the Giemsa intensely, and resembling in every way the familiar hyaline droplets of degenerating renal cells. These largest droplets accept the acid fuchsin in the Altmann-Bensley stain, but I have gained the impression that they arise from the argentophile droplets rather than from a breaking-up and fusion of the mitochondrial structures.



18



19



20

Fig. 18 Rat kidney. Two small collecting tubules with delicate supranuclear filaments. Cajal.

Fig. 19 Rat kidney. Uranium nitrate, Nephritis. Proximal convoluted tubule containing hyalin droplets with argentophile granule. Cajal.

Fig. 20 Frog's kidney. Tubules showing various types of argentophile structures. Cajal.

Fahr (52) has also recently made the observation that this type of 'gross-trophige' degeneration may be produced experimentally in the rabbit's kidney with uranium nitrate, and is disinclined to derive the droplets from the mitochondria of the cell.

The appearances observed in the epithelial cells of the frog's kidney are also very puzzling and difficult to interpret. In the glomeruli, a concentrated juxta-nuclear mass is present in all the cells, identical with that described in the rat's kidney.

In the proximal portion of the convoluted tubule there is found to either side of the nucleus, but rather nearer the distal pole, a small irregular, granular filamentous or ring-shaped mass, which is quite definitely the homologue of the Golgi apparatus of other cells. Such an epithelial cell, cut in a plane parallel to the basement membrane, shows the nucleus surrounded by a ring of discrete masses, which do not form a continuous skein, but are interrupted (fig. 22A).

In frogs injected with trypan-blue this type of apparatus is present in the cells containing the granules of dye, which in general occupy the supra-nuclear zone of the cytoplasm. The dye granules and the argentophile bodies are quite unrelated.

In some of my preparations, the cell boundaries are sharply impregnated by the silver, appearing as delicate black lines. The striated border is also sharply brought out (fig. 22).

In another part of the tubule, probably the distal portion of the convoluted tubule or Schaltstück, which contains no blue staining granules, the argentophile bodies are in the form of rounded globules or granules, varying slightly in size and intensity of staining, and occupying a zone in the middle nuclear plane (fig. 22B). The homology of these granules to the Golgi apparatus is not clear, and it is possible that they represent excretory substances of some sort, possibly chlorides or phosphates (Leschke (109)).

Finally, in still another portion of the tubules (corresponding to the Henle loop) there is obtained an excellent impregnation of the basal filaments and mitochondria (fig. 22 C). The cytoplasm in the supranuclear zone is somewhat more intensely stained,

but no definite structures comparable to the Golgi apparatus of other cells is brought out.

This description corresponds to the appearances usually observed in the frog's kidney. Some of our preparations, however, show curious structures of very different type, the nature of which is entirely obscure. These are limited to certain portions of the secretory tubules—probably the distal convoluted portion or *Schaltstück*, although it is not possible to be sure of this.

They consist of sheaves of filaments, often of great length, sometimes beaded or with nodular varicosities. They run either perpendicular to the basement membrane, along the lateral aspects of the nucleus, or in some instances, lie above the nucleus and have a course more or less parallel to the basement membrane (figs. 20–21).

These bundles of fibrils do not appear to form closed skeins or to anastomose with one another, but overlies and cross. The individual fibrils are often irregularly fusiform, with tapering ends. They may be quite rigid, almost crystalline in appearance, or more wavy and filamentous. Scattered amongst them are small isolated clumps and granules of varying size.

b. In ordinary connective tissue cells. A small juxta-nuclear apparatus has been described by v. Bergen (10), Cajal, Deinecke (47) and Kolster (102); in endothelial cells by v. Bergen (10) and by Cajal (28); in smooth muscle cells of blood vessels by v. Bergen (10); and in various types of wandering cells by v. Bergen (10), Verson (179) Maccabruni (115), Barinnetti (6) (Plasma-cells—relation to centrosome, '12) and Fananas (55).

In *cartilage cells*, Pensa (136) first described a distributed apparatus which he considered to resemble the diffuse net found by Golgi in the ganglion cell. Later v. Bergen showed that the Golgi apparatus in cartilage cells, as in most other non-nervous elements, was limited to the juxta-nuclear region, and interpreted Pensa's diffuse apparatus as a chondriom. Pensa (137) has accepted this correction, and v. Smirnow (165), Barinnetti (6) and Kolster (102) are in agreement with v. Bergen on this



Fig. 21 Frog's kidney. Tubules showing argentophile filaments. Golgi.

Fig. 22 Frog's kidney. A—Proximal convoluted tubule. Golgi apparatus in the form of discrete peri-nuclear filaments, rings and loops. B—Tubule containing argentophile droplets. C—tubule showing impregnation of basal-rod.

point. Comes (40, 41) still claims that Golgi net and mitochondria in cartilage cells are identical structures.

The controversy is interesting, because it has brought out the point that the silver method is not always specific, and that under certain conditions the mitochondria may be impregnated. This occurs regularly, as I have mentioned, in the Henle tubules of the kidney.

Both Pensa (136) and Cajal (31) have described an interesting series of changes in the zone of growing cartilage adjoining the line of ossification. Following the enlargement of the cells the net loses its localized character, hypertrophies and finally, with the degeneration of the cartilage cell, undergoes granular disintegration.

Cajal (31) seems to have been the only one to study the behavior of the Golgi apparatus in *osteoblasts*. During the period of functional activity, the net is very well developed, and large, usually occupying that part of the cell directed towards the osteoid tissue. In the finished bone corpuscle, the net shrinks to a small compact mass.

Teeth. Although Massenti (123), using pig embryos, had previously described a Golgi apparatus in the form of a large skein occupying almost the entire cytoplasm of the pulp cells and odontoblasts Cajal (31) depicts structures of a more typical character and localization. As the odontoblast becomes differentiated from the connective tissue elements of the pulp, the net increases in size, and comes to form a large oval rather granular mass, occupying the supra-nuclear portion of the cytoplasm. The further fate of the structure could not be followed, since decalcification interferes with the reaction, but Cajal regards the hypertrophy during the secretory phase of the odontoblast as another example of the cyclical changes seen in goblet cells, growing cartilage cells, etc.

Muscle fibers. There are a number of papers dealing with the endo-cellular reticulum of striated muscle fibers. The earliest is that of Cajal (26), in which he found in the wing muscle of certain insects a network continuous with the ramifications of the tracheal tubes, and therefore probably a true cana-

licular system. This was confirmed by Fusari in 1894 for mammalian muscle and by Veratti (178) in 1902. Veratti, however, denied that the endocellular apparatus in insects represented a continuation of the tracheal tubes, and regarded it as composed of solid filaments. Sanchez (155), a pupil of Cajal, confirmed the tracheal origin of the reticulum in insects, looking upon it as a tubular apparatus, probably of importance for the nutrition of the fibers. The system is composed of transverse meshes on either side of the 'bandes claires,' united by longitudinal connections.

Two other recent papers, one by Martinotti (122), the other by Fananas (54) have not been available.

Quite different is the apparatus described by Luna (113) in the cardiac muscle fibers. Here he finds granules, rods, curved filaments or more complete nets lying at one or both poles of the nucleus. He believes that the granules and rods might possibly be mitochondrial.

c. Gonads. The studies upon the gonads are of much more theoretical interest, because of their bearing upon the question as to whether the Golgi apparatus is a permanent structure, and a heritable constituent of the cytoplasm, or whether it is more ephemeral in character, and related to the vegetative activities of the cell. It is also in the sperm cells that the topographical relation of the net to the centrosphere is most evident, so that the structure during spermatogenesis might be expected to show interesting modifications.

Platner (145), a number of years before the discovery of the Golgi apparatus, had described a structure surrounding the centrosomes of the spermatogonia, which he called a Nebenkern. Heidenhain (68) in 1900, in the sperm cells of *Proteus*, found with the iron hematoxylin stain, an incomplete basket-work surrounding the centriole. Heidenhain used the term Zentral-kapsel or pseudo-chromosomes for these bodies, and believed that they were formed by special differentiation from the Benda mitochondria. This view finds support in the recent observations of Chambers (33) upon spermatogenesis in the grasshopper, in

which the mitochondria, vitally stained with Janus green, appeared to enter directly into the formation of the Nebenkern.

Sjövall (164), using a modification of the Kopsch method, demonstrated these structures in the spermatocytes, spermatogonia and spermatids of the white mouse, and concluded that Heidenhain's view of their mitochondrial origin was erroneous. Benda, and especially Weigl (180), have also taken this stand. Sjövall also found a net in the Sertoli cells.

Perroncito (140, 141), using the Golgi method, carried the observations of Sjövall a step further, by describing the alterations of the net during the maturation divisions. He finds in *Paludina*, that in the prophase, the net breaks up into granular fragments, which he calls dittosomes, and which are distributed equally to the two daughter cells. The skeins are then reformed from the granules, and in the spermatids come to occupy their usual juxta-nuclear position. This process of fragmentation and distribution during mitosis he calls dittokinesis.

The fate of the Golgi apparatus in the adult spermatozoa is unknown, nor has it been established that the substance of which it is composed enters the egg during fertilization. The phenomenon of dittokinesis, however, is established, not only for the sperm cells, but also for the somatic cells (fig. 4). Deinecke (47) ('12) has given a very clear description of this process in the flat cells of Descemet's membrane, where the net forms in the resting cell a thick skein of interwoven and anastomosing threads. During karyokinesis the skein becomes looser and gradually surrounds the nucleus, the individual threads grow thicker, lose their connection and break up into unequal bent fragments which are heaped up at the poles of the nucleus. During anaphase they change into short thick rods and granules surrounding the nuclei, and lying chiefly in the equatorial plane. The size of these dittosomes is only slightly smaller than that of the chromosomes, their number somewhat greater. With the formation of the diaster, the dittosomes surround the daughter chromosomes, being more densely aggregated at both poles. The region of the spindle remains free of them.

The new nets are formed by a fusion or sticking together of the granules or rods, but may remain discreet for a time. In this way, it is possible to recognize a recent mitosis, even after the nuclei have reformed.

During the monaster stage one sees often a pairing of the granules and double rods. Whether this indicates a splitting of the dittosomes comparable to that of the chromosomes, Deineke leaves undecided. At any rate, the above series of changes unquestionably leads to an even distribution of the mass to the two daughter cells.

Studies of the Golgi apparatus in the female gonads have been made by Sjövall (164), Weigl (180), Cattaneo (32), and Kulesch (104), and Weigl (180) and Hirschler (70) in the oocytes of invertebrates; in the primitive germ-cells of 3-4 day chick embryos by v. Behrenberg-Gossler (16). All these writers are in substantial agreement as to the main facts namely, that in the young oocytes and in the follicle cells, there is a circumscribed net at one pole of the nucleus, which in the ripe ovum breaks up into filaments and granules (or, according to Kulesch, small irregularly angular rings, bent threads and discs) which are with difficulty distinguished from the mitochondria and other cytoplasmic granulations.

V. THE GOLGI APPARATUS IN EMBRYONAL CELLS

The recognition by Golgi (64), Fananas (54) and Cajal (31) that the Golgi apparatus is present in all types of cells, even at a very early stage of development (chick embryos of 30-40 hours—Cajal) seems to establish firmly the principle that the structure is an important and constant component of the cell.

In many of these fetal cells—the mesenchyme—the endothelium of the pericardium and of the primitive blood spaces—the cells of the Wollfian ducts and the entoderm of the intestinal tract, the neuroblasts, and even the erythroblasts and wandering cells, the apparatus is highly typical and constant in its relation to the centrosphere.

The attempt has been made by Fananas to trace the development of the net from granules and batonnets in the cytoplasm.

As Cajal (31) points out, however, the not infrequent impregnation of the mitochondria and of skeletal and sustentacular structures in early embryonic cells makes such an interpretation doubtful.

One general principle can be deduced from a study of the Golgi apparatus in embryonic tissue, and that is the definite polarity of the structure in all fixed non-mobile cells. The location of the net in every case, as Cajal has pointed out, is such that it occupies the 'external' part of the cell,—that is, the portion above the nucleus directed originally towards the free surface, and opposite that pole which is towards the interstitial tissue and the nutrient supply. This polarity is preserved in adult life in the case of epithelial cells lining ducts and cavities, but may be lost in the case of solid glandular organ or tissues which undergo profound modification and derangement during development. The significance of this polarity which would seem to be bound up with the relation of the Golgi net to the centrosphere, is by no means clear, but it seems to be one of the most fundamental and most striking characteristics.

VI. THE GOLGI APPARATUS IN PROTOZOA

The literature contains but one reference to the occurrence of the Golgi apparatus in Protozoa. Hirschler (70) describes in the cytoplasm of *Monocystis ascidia*, a Gregarine parasite of the ascidian *Ciona intestinalis*, diffusely scattered ring and half ring forms, demonstrable by prolonged exposure to 2 per cent osmic acid and resistant to turpentine (Sjövall's modification of the Kopsch method). Whether these structures are the homologues of the Golgi apparatus of the metazoan cell, needs further study.

VII. THE GOLGI APPARATUS UNDER PATHOLOGICAL CONDITIONS

The modifications which the Golgi apparatus undergo under pathological conditions have been little studied, and, so far, have not added any new suggestions as to the real nature of the structure.

In the cells of malignant growths, Golgi nets, often atypical, have been found by Moriani (125) in a human breast carcinoma, by Veratti (177) in a transplantable mouse cancer; by Lucioni (112) in a naevus; by Savagnone (157) in carcinoma of the breast, in a sarcoma of the jaw and in a giant-celled sarcoma; by Tello (172) in carcinoma and adenoma, in epithelioma and in experimental granuloma caused by Kieselguhr injections. Tello studied especially the distribution of the net in foreign body giant cells, where there are multiple nets—one usually in relation to each nucleus. In tuberculous giant cells, on the other hand, as shown by Fananas (55), the net is usually centrally placed, in relation to the multiple centrosomes.

Regressive changes (fragmentation, pulverization, etc.) in the Golgi net have been described by Fananas (55) in caseating giant cells; by Marcora (117) in the ganglion cells of the hypoglossal nucleus, following avulsion or section of the nerve; by Battistessa (8) in the ganglion cells of animals poisoned by lead or strychnin; by San Giorgi (156) in toxic nephritis, and by Del Rio Hortega (97) in the ganglion cells of a case of paralytic rabies.

Very interesting are the recent experimental studies of Cajal (31) dealing with the effect of traumatic injury of the nerves upon the Golgi apparatus.

An incision of the central nervous tissue brings about complete destruction of the net only in those ganglion cells most severely injured by the trauma. The apparatus of cells near the line of incision, though perhaps slightly compressed or deformed, shows no grave disorganization. This would indicate a considerable fixity of structure, and firmness of texture, since, were the impregnated substance of fluid consistence, one would expect to find it dispersed through the cytoplasm or confluescing into larger droplets.

Cajal has further established the fact that section of a peripheral motor nerve had no effect upon the structure of the Golgi apparatus in the central ganglion cell. There does occur a degeneration of the apparatus in the cell of the sheath of Schwann, distal to the section.

There are thus very few controlled studies of the alterations of the net under experimental conditions, and it seems that something further should be added to our knowledge in that way. The great difficulty had been, and will be, the capricious behavior of impregnation methods; until simpler and more reliable methods shall have been discovered, the interpretation of slight variation in the morphology of the structures will always be open to considerable suspicion.

VIII. GENERAL CONSIDERATIONS

This completes the list of cells and tissues in which structures of this type have been demonstrated. They may be considered as universally present in every type of cell, although the variety of form which they assume at once brings up the query as to whether they are all homologous structures. That, I think, is almost impossible to answer until we know something of their function and significance. Morphologically there seems to be no single character by which we can group them altogether. The staining reactions are probably not entirely specific; we have found instances—as for example in the Henle tubules and in cartilage cells—in which mitochondrial structures are more or less regularly impregnated by the silver methods.

In many types of cells in which the location of the centrosome is known, there is, as Barinetti (6) insists, a topographical relation between Golgi net and cytocentrum. But such a relation cannot be established for the ganglion cells, in which the net completely encircles the nucleus, nor for the muscle cell, nor for the cells of the choroid plexus, still less for the cells of the convoluted tubules, or for the liver cells. So that this criterion is not universally applicable, at least to fully differentiated and highly specialized types of cells.

Much of the discussion about the nature and homologies of these things has hinged about the question as to whether they are solid, that is fibrillary; or canals filled with fluid, and made to appear as solid filaments by the metallic impregnation methods. With the canalicular theory the name of Holmgren is associated, and though there are many shades of opinion in

regard to detail the general idea that these nets and filaments represent canals with or without definite walls, has had the support of such expert histologists as Studnicka (170), Retzius (149), Cajal (31), and, in this country, of Bensley (14) and Cowdry (42). Holmgren was not the first to observe 'endocellular canals' or 'Saftkanälchen.' As far back as 1887 Nansen (129) described in the protoplasm of nerve cells of *Homarus* and in the spinal ganglion cells of *Myxina glutinosa*, primitive tubes consisting of hyalin contents enveloped in sheathes of spongioplasm. These were probably identical with the Saftkanälchen. Nelis (132) also described an 'état spirémateux' in the cytoplasm of certain mammalian nerve cells. He speaks of 'bandes incolores' of about the same diameter, sometimes straight, sometimes convoluted which did not branch, and therefore formed no reticulum. This spireme was not constantly found, and does not seem to be the same thing as the Golgi net.

Holmgren (73) made his first contribution to this subject in 1899, a year after Golgi's first paper, and without, apparently, knowing of the work of Nansen and Nelis. In the spinal ganglion cells of rabbits and of *Lophius piscatorius* he found an endocellular system of canals communicating with the pericellular lymph spaces; and in the following year he published a large monograph on the ganglion cells of *Lophius* (72). His earlier views, expressed in these papers, that the endocellular Saftkanälchen are to be regarded as lymph channels, and are thus continuous with structures of connective tissue origin, were later abandoned by him.

Morphologically Holmgren considered his canaliculi to be identical with the Golgi net, basing his opinion upon a study of his own preparations, and those of Retzius, prepared according to the earlier Golgi technique.

In 1900 (76), in a study of the ganglion cells of *Helix pomatia* Holmgren described a penetration of the nerve cells by cell-processes from surrounding cells. This view was developed in subsequent studies. The penetrating cells of neuroglial origin he called trophocytes, and to the network formed by the penetrating cell processes, he applied the term trophospongium.

These prolongations of the trophocytes, he believed, became canalized by a vacuolization of their cytoplasm, the confluence of the vacuoles giving origin to the canal. This was an irreversible process, but the cell prolongation was capable of amoeboid motion within the host cell.

Studies on various tissue cells, which I shall not review in detail, confirmed him in his view, and lead him to the following generalization. The cells of the body are of two orders of physiological dignity—high and low. Those of exalted function are the nerve cells, muscle cells, sex cells, certain glandular cells. The lower order of cells, which are the trophocytes, function as servitors, looking after the wants of their more specialized neighbours by means of their trophospongia.

Although this hypothesis is vaguely expressed, and open to obvious criticism, Holmgren has maintained it in a long series of papers (77–96), many of them controversial, and adding no new evidence. The arguments against any such generalized conception are apparent enough. To what order shall we assign the leucocytes and other wandering cells? Where are the trophocytes of cartilage cells? Why have the trophocytes about the ganglion cells, as well as all the other types of cells classed with the lower order, endocellular nets, and what cells look after their lowly wants?

Holmgren has always insisted vigorously upon the identity of his trophospongium with the Golgi apparatus; on the other hand, all who have worked with the Golgi or Cajal methods deny that the structures which they bring out reach the surface of the cells or communicate with other cells. Even Cajal, who regards the Golgi structures as canaliculi, believes them to be wholly endocellular, except perhaps in the special case of the insect muscles, in which the homology with the Golgi net is not very clear at best.

Ross (153) in a recent paper on the trophospongium of the ganglion cells of the crayfish, describes the penetration of the cytoplasm by partitions and fibrils from the surrounding neuroglia, but rejects the idea that these have any relation to the internal reticular apparatus.

This view, I think, may be accepted without reserve; nor does it seem that Holmgren's generalizations are based on sound evidence, nor that they have added much of value to the subject.

Leaving aside, then, this controversial phase of the subject, one may ask what can be said as to the more intimate physical structure of the Golgi apparatus. It seems to the writer, that the conceptions of Cajal (31) best meet the observed and established facts. Cajal's view is that the apparatus represents a canalicular system, filled with a lipoid-containing substance which reacts to the specific impregnation methods employed. The walls of this system are presumably fairly fixed and rigid in cells of a sedentary habitus, and permanent form, but more plastic in secretory cells and in young cells frequently undergoing mitosis.

It seems probable that the quantitative changes observed during activity indicate a using up of a store of stainable material within these canaliculi, and that the re-appearance of the net during the quiescent stage is due to the re-accumulation of the substance within more or less preformed and permanent channels. What purpose this material serves in the cell metabolism, and what is its more intimate chemical structure, are questions unanswerable with the data at hand.

IX. SUMMARY AND CONCLUSIONS

There is present in the somatic and sex cells of all metazoa, and possibly also, of protozoa, a cytoplasmic structure of considerable complexity and size. demonstrable by prolonged fixation in osmic acid, or by silver impregnation and reduction. The reaction of this structure to osmic acid indicates, of course, a lipoid component, but there are no other data bearing upon its chemical composition. Nor is anything certain known of its physical characters. Its invisibility in the living cell would indicate a low refractive index. The fundamental question as to whether the impregnated structures are canalicular or filamentous remains unsolved. The constant topography in many types of cells, particularly the definite relation to the cytocentrum would favor the idea that the structures are at least in part

solid, rather than casual rifts or fluid-filled canals in the cytoplasm. The fragmentation or dispersion of the net which occurs during certain secretory phases, or accompanying pathological changes in the cell, and particularly during cell division, would also suggest a solid or semi-solid consistence.

The Golgi apparatus in the secretory portion of the renal tubules does not conform to the usual closed skein found in many types of glandular epithelial cells, but is dispersed and assumes complex and varying forms. In the glomerular cells, on the other hand, and in the epithelium of the collecting tubule and of the pelvis the structure is more typical both in form and location.

Injury to the epithelial cells of the convoluted tubules (uranium nitrate poisoning) is followed by complete disintegration and disappearance of the Golgi apparatus. This appears to precede the complete necrosis of the cell. The large hyalin droplets found during the degeneration of the cells contain an argentophile component possibly derived from the remains of the Golgi apparatus.

The structures brought out by the Golgi or Cajal technique are more resistant to autolysis than are the mitochondria.

There occurs regularly in the rat's kidney an elective impregnation of the mitochondrial filaments of certain portions of the Henle loops. This illustrates the fact that the method is not absolutely specific.

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ON THE FORM AND ARRANGEMENT IN FASCICULI OF STRIATED VOLUNTARY MUSCLE FIBERS

A PRELIMINARY REPORT

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FOUR FIGURES

In the last few decades, relatively little special attention has been given to the form of striated voluntary muscle fibers and to their arrangement in the fasciculi. In the anatomic literature of this period, consideration is given mainly to the structure of the myofibrils, to the relation of the connective tissue of the muscle to the fibers, to the development of muscle fibers and the development of the musculature as a whole. One studying current texts is impressed with the unanimity of expressed views concerning the form of muscle fibers and the question is treated as having received satisfactory solution.

Heidanhain¹ in 'Plasma und Zelle' treats of the form and length of voluntary muscle fibers as follows:

Da der Gegenstand allgemein bekannt ist, können wir uns kurz fassen. Es handelt sich um lange faserförmige Gebilde, welche in kleinen Muskeln von einem Sehnenende bis zum anderen hindurchlaufen und an diesem immer abgestumpft enden, im Inneren sehr grosser Muskeln hingegen auch frei und zwar unter allmählicher Verschmälung mit spitzen Enden auslaufen. Sie werden bei geringer Breite (9–60 μ) bis zu 12 cm. lang und daher findet man nur in Muskeln, welche, parallel der Faserung gemessen, die Ausdehnung von 12 cm. überschreiten, die erwähnten freien Endigungen.

This quotation expresses fairly well, I believe, the current views of the form and mode of ending of striated voluntary muscle fibers.

¹ Heidanhain, M., *Plasma und Zelle*. Fischer, Jean, 1911, p. 529.

Bardeen,² as a result of a study of teased preparations made from the external oblique of certain mammals, deviates from the current views and gives a much more correct statement as concerns the form and relations of striated voluntary muscle fibers; indeed his brief statement is one of the most accurate I have found and is here given in full. His words read as follows:

The individual muscle-fibres either run from one tendon to another or they may end at one extremity or at both within the muscle fasciculi which extend from tendon to tendon. We may therefore distinguish two modes of ending of individual muscle-fibres: the '*intratendinous*', where the tip of the fibre terminates within a definite extension of a well marked tendon; and the '*intrafascicular*,' where the muscle-fibre terminates in the midst of a bundle of other muscle fibres which have a different region of termination. In the former case the muscle-fibre has a rounded or cone-shaped termination, often swollen in isolated specimens. In the intrafascicular mode of ending the muscle-fibre gradually becomes more and more narrow until it terminates in a thread-like extremity.

In Bardeen's figures 2 and 4, types of these modes of ending and of the form of muscle-fibers are given, figure 4 including spindle-shaped fibers. Bardeen's statement of the form and mode of ending of muscle fibers, however, is antedated by the account given by v. Kölliker in his *Handbuch der Gewebelehre des Menschen*, which may be added to the references here given. Kölliker's³ account reads as follows:

Ueber die Gestalt der Muskelfasern haben besonders die Untersuchungen von Herzig und Biesiadecki, dann von mir, W. Krause, Weismann, Aebly und Kühne Aufschluss gegeben. Nach diesen Erfahrungen kann es wohl als Regel bezeichnet werden, dass die Muskelfasern im Innern grösserer Muskeln spindelförmig sind, die an den Enden dagegen ein inneres spitzen und ein in die Sehne übergehendes breites Ende besitzen, welches entweder abgerundet ist oder in einige stumpfe Spitzen ausläuft oder auch wie treppenförmige Absätze darbietet. Ausser spindelförmigen Fasern kommen im Innern der Muskeln noch manche andere Formen vor, am gewöhnlichsten an dem einen oder an beiden Enden stumpfe Fasern.

² Bardeen, C. R., Variations in the internal architecture of the m. Obliquus Abdominis Externus in certain mammals. *Anat. Anz.*, vol. 23, 1903.

³ v. Kölliker, A., *Handbuch der Gewebelehre des Menschen*. Erster Band. Engelmann, Leipzig, 1889, p. 371.

Kölliker quotes E. H. Weber (the original I have been unable to find) as regarding spindle-shaped fibers as the prevalent form of striated voluntary muscle fibers.

It is not my purpose at the present time to enter upon a more extended discussion of the literature dealing with the form and arrangement in fasciculi of striated voluntary muscle fibers. It is hoped that the quotations given may suffice to orient the results here to be presented. In the course of this brief report other pertinent literature will be considered as occasion demands.

My own studies on the form of striated muscle fibers have been made largely on teased preparations. The muscular tissue was obtained largely from adult rabbits. The maceration preparatory to teasing was by the hydrochloric acid method developed in this laboratory.⁴ The method as used for the maceration of muscular tissue may be given here in detail in the hope that other workers may feel tempted to make use of it in a further analysis of this tissue, since a correct understanding of the form and arrangement of muscle fibers in fasciculi, as also their length, is of importance in valuating certain fundamental conceptions concerning the functions of muscles. For instance, according to E. Weber's law of the working capacity of a muscle we are taught that the lifting power of a muscle is proportionate to the cross section of its fibers or fasciculi when arranged parallel, while the extent of elevation is proportionate to the length of its fibers.

The method as used is as follows:

After freely bleeding an adult rabbit a cannula was inserted into one of the iliacs central to the inguinal ligament or into the subclavian before it passes under the clavicle and firmly secured by ligature. A 75 per cent solution of hydrochloric acid was then quickly injected at a pressure of 25 to 30 pounds. The apparatus used in obtaining and maintaining pressure was described by the author in the *Am. Jour. Anat.*, vol. 6.⁵ It is desirable to have the acid injected enter the tissues as quickly and as freely as possible. The pressure is maintained for several minutes. Some 15 to 20 minutes after the injection is com-

⁴ Huber, G. Carl, A method for isolating the renal tubules of mammals. *Anat. Rec.*, vol. 5, 1911.

⁵ Huber, G. Carl, The arteriolae rectae of the mammalian kidney. *Am. Jour. Anat.*, vol. 6, 1907.

pleted, the muscles are exposed and separated and removed and placed in a 75 per cent solution of hydrochloric acid. In removing a muscle care should be taken to remove the entire muscle, at least portions extending from tendon to tendon and great care should be taken not to crush the muscle during removal. The muscle pieces or entire muscles remain in the hydrochloric acid for about 3 hours, the period varying a little, depending on the thoroughness of the preliminary injection. After thorough maceration is obtained, the acid is carefully poured off and distilled water slowly added. The water is renewed at frequent intervals until it is practically free from acid. In the distilled water the muscle pieces remain about 24 hours, though a stay of 4 to 6 days is not harmful. After thorough washing in distilled water the larger pieces are usually readily broken up into smaller bundles of fasciculi. Small bundles of fasciculi are now transferred to a hemalum solution diluted to one half with distilled water. The transfer from the distilled water to the hemalum solution should be executed with care if one wishes to obtain fasciculi through their entire length. The transfer is best made with a glass rod, lifting the small bundle carefully as it leaves the water. In the hematoxylin solution the small bundles remain about 24 hours. This interrupts the maceration and stains the fibers. They may be kept indefinitely in the hematoxylin solution and are best stored in this solution for future use. In this solution the muscle bundles become quite hard and brittle and contract to about two-thirds or even to one-half of their former length. The preliminary teasing I have carried on in Esmarch dishes under the stereoscopic binocular and in a 0.5 per cent solution of ammonia water. In the ammonia water the stain attains a purple-blue color and the hard and brittle bundles become soft and pliable. A stay of one half hour to one hour in the ammonia water prepares the bundles for preliminary teasing. In the Esmarch dishes the bundles of fasciculi are with care separated into separate fasciculi. It should be stated, however, that a fasciculus is not a unit of muscle structure. For a distance all of the fasciculi of a bundle are readily separated. However, at one or several points small bundles of fibers or single fibers pass from one fasciculus to contiguous fasciculi. Great care is thus necessary and very careful teasing to completely separate what is known as a fasciculus. Bardeen² has noted the fact that muscle fasciculi are joined by fibers. After the preliminary teasing resulting in the separation of a single fasciculus, the final teasing is undertaken on a large slide or lantern slide cover prepared as follows. The slide is thoroughly cleaned in acids and alcohol and wiped dry. Narrow strips of wax plates (the plates used in wax reconstructions) 2 mm. thick are cut and placed near the borders of the slide in the form of an oblong and pressed to the slide. The slide is then gently heated until the wax strips adhere. The slide on cooling is ready for use. The shallow well thus formed is filled with ammonia water and an isolated muscle fasciculus transferred to it. The final teasing may then be undertaken. It has repeatedly been possible to separate completely all or nearly all of the muscle fibers of a given fasciculus, even with fasciculi having a length of about 6 cm. The teased fibers may then be arranged in their approximate positions. Only when this has been accomplished can a muscle fasciculus be considered as having been teased. A worker should not attempt this unless he has at his disposal some 3 to 4 uninterrupted hours, and ought to bear in mind that the best results are obtained by 'making haste slowly.' The mounting of such preparations presents many difficulties and discouragements. My procedure

is as follows: A fasciculus is teased until nearly all its fibers have been separated. The ammonia water is then very slowly and carefully withdrawn by means of a small dropper with point drawn to a capillary tube. This is undertaken under the binocular, observing the effects of currents. The water is withdrawn until only a thin layer remains, only sufficient to enable moving the fibers on the slide. The final teasing and arranging of fibers may now be undertaken. As the ammonia water evaporates, the muscle fibers begin to adhere to the slide. The wax wall may now be removed and a large cover glass, on the under side of which a thin layer of glycerin has been spread, is gently lowered over the preparation from one edge. It is necessary to obtain the right degree of drying in order to gain successfully mounted preparations. If not sufficiently adherent to the slide the muscle fibers will move, float and break. If allowed to dry too much the muscle fibers, although fixed in place, will appear fragmented. Such preparations are not valueless since the fragments of the fibers are not displaced laterally. Thus a single fiber may readily be traced throughout its whole length.

By this method of preparation the muscle fibers show only faint cross striations, though they present a blue color. The nuclei are not evident. Neither has it been possible to locate the place of entrance of the nerve fibers. The sarcolemma seems very resistant to the acid, the neurolemma less so. In the ammonia water the muscle fasciculi appear to regain the length they had prior to staining in the hematoxylin solution. The exact relation existing between the lengths as presented by teased fibers and living muscle fibers I am unable to determine definitely.

The drawings here presented were made from preparations of muscle fasciculi teased completely, and arranged on the slide in their approximate positions, approximate with reference to the ends of the fasciculus teased. The drawings were made with the aid of the camera lucida at a magnification of 50 diameters, and are reduced 10 times in reproduction. The length of the respective fibers is accurately given. The thickness of the fibers is correctly given as pertains to the thicker portions of the fibers. At the attenuated ends the ink lines follow the outer border of the pencil outlines.

The results of these observations may be briefly recorded as follows:

It is usually stated that in muscles having relatively short fasciculi the muscle fibers extend from tendon to tendon. This is of course not determined by the size and length of the muscle as a whole since in semipinnate, pinnate and compound pinnate muscles and in muscles where distal and proximal tendons overlap the lengths of the respective fasciculi of a given muscle are much shorter than the length of the muscle as a whole.

In figure 1 are presented some muscle fibers teased from fasciculi taken from the gastrocnemius of an adult rabbit. The fibers in group A, drawn from a completely teased fasciculus taken from the proximal portion of this muscle have an actual length of 1 cm. (these and all measurements given are obtained by dividing the length of the respective fiber as measured in



Fig. 1 Muscle fibers from the gastrocnemius of an adult rabbit. Group A, actual length 1 cm., from fasciculus taken from the more proximal portion of the muscle. Group B, actual length 1.5 cm., from fasciculus taken from the more distal portion of the muscle. $\times 5$.

the drawing by 50, the drawing having been made at a magnification of 50 diameters). The fibers in group B have an actual length of 1.5 cm. and are from a completely teased fasciculus taken from the more distal part of the same muscle. In all of the fasciculi from this muscle completely teased, the great majority of the muscle fibers extend from end to end or from tendinous insertion to tendinous insertion. In material prepared as above described, at the place of termination of a muscle fiber

in tendon, the end of the muscle fiber stains more deeply in hematoxylin than does the same fiber in close proximity. The true end of the fiber differs in appearance from the end of a broken fiber. The tendon ends of various fibers vary slightly in shape. They may appear as if cut at right angle to the fiber, as slightly beveled, as slightly rounded, tapering a little or having the form of a blunt cone and now and then as slightly expanded, though this may be due to a slight flattening of the end of the fiber. Now and then tendon ends of muscle fibers are met with that give the impression as though the sarcolemma did not enclose the end but terminated ring-shaped at the extreme tendon end of the respective fiber, but the limitations of the method used are such that this question could not be conclusively decided. In this connection it is of interest to note the observations of O. Schultze,⁶ who believes that muscle fibrils and tendon fibrils are parts of a single structure but this observer adds that the behavior of the sarcolemma at the ends of the fibers deserves further study. Also the studies of Baldwin,⁷ who regards the sarcolemma as covering the tendon ends of muscle fibers and denies the continuity of muscle fibrillae and tendon fibrillae, and discusses two types of terminations of muscle fibers in tendon; one type in which the long axis of muscle and tendon fibers coincide, the other type in which they meet at an angle. In the former the tendon fibrils are attached to cone shaped processes of the sarcolemma dovetailed into the tendon ends; in the latter type the sarcolemma end is considerably thickened and presents a number of projections into the muscle substance. Digitations or branchings of muscle ends or step formations have not been observed by me in my teased preparations. It should be understood, however, that in successfully macerated preparations the collagenous connective tissue is so completely removed that it is not evident on teasing. Out of quite a number of fasciculi with fibers of type B, of figure 1,

⁶ Schultze, O., Über den direkten Zusammenhang von Muskelfibrillen und Sehnenfibrillen. Arch. f. Mik. Anat., vol. 79, 1912.

⁷ Baldwin, W. M., The relation of muscle fibrillae to tendon fibrillae in voluntary striped muscle of vertebrates. Morph. Jahrb., vol. 45, 1913.

successfully and completely teased, in only two and in each only one fiber was found which did not extend from tendon end to tendon end. In both of these fibers one end reached the tendon, terminating as adjacent fibers, while the other end reached to about the middle of the fasciculus ending in a fine tapering filament. The fibers in a number of fasciculi having muscle fibers of the length of type B were counted and averaged about 20 fibers to a fasciculus.

What length a muscle fasciculus of an adult rabbit may attain and still have the great majority of its fibers reach from end to end is a question I am at present unable to answer definitely. Of the muscles teased, none in which the contained fasciculi reached a length of a little over 2.5 cm. did I find such in which the majority of the muscle fibers reached from end to end. However, samples have not been taken from nearly all of the muscles and it may be that in certain of them fasciculi having a length of over 2.5 cm. in which the majority of the fibers extend from end to end, may be found.

In figure 2 are presented type fibers obtained from a completely teased and successfully mounted fasciculus, taken from one of the adductor muscles of the thigh of an adult rabbit. In this fasciculus a single muscle fiber (A) extends from end to end or from tendon insertion to tendon insertion; both extremities showing the characteristic staining and appearance of the tendinous end of a muscle fiber. This fiber has an actual length of 3.64 cm., a length which is regarded as the length of the fasciculus. After final teasing and after withdrawing the ammonia water

Fig. 2 Muscle fibers from the thigh adductor of an adult rabbit. Teased fasciculus had a length of about 3.5 cm. The completely teased fibers are in the drawing placed with reference to the ends of the fasciculus. Fiber A, has actual length of 3.64 cm.; a, 2.1 cm.; b, 1.8 cm.; c, 1.5 cm.; d, 1.3 cm.; e, 2.1 cm.; f, 1.9 cm.; g, 1.7 cm. $\times 5$.

Fig. 3 Types of muscle fibers teased from a single muscle fasciculus, having a length of a little over 4 cm., taken from one of the larger thigh muscles of a rabbit. This fasciculus contained 37 fibers. The fibers are arranged with reference to an imaginary line, bottom of figure. The tendon ends of fibers ending intrafascicular are brought to this line. Certain of the fibers sketched have an actual length as follows: a, 2.9 cm.; b, 2.4 cm.; c, 1.92 cm.; d, 0.9 cm.; e, 1.4 cm.; f, 0.14 cm.; g, 2.9 cm.; h, 3.04 cm.; i and j, 2.9 cm. $\times 5$.

c A a



from the well on the slide, as explained in the detailing of the method used, I was able to arrange the teased fibers so as to have the tendon ends of the teased fibers reach imaginary lines drawn at right angles to the ends of the single muscle fiber which extends from end to end in this fasciculus. The spindle-shaped fibers hold approximately the same relative position with reference to the ends of the fasciculus as before teasing as was determined at the time of teasing. This fasciculus, completely teased, contains 26 muscle fibers, of which as stated one passes from end to end, 10 others reach one tendon end, 12 the other tendon end and 3 are spindle shaped fibers reaching neither tendon end. Of the 26 fibers, 15 type fibers are given in figure 2. This bundle of fibers completely teased is here spoken of as a fasciculus. I have above stated that fasciculi are not units of structure, but that from each small bundles of fibers or single fibers pass from one fasciculus to contiguous fasciculi. A single 'fasciculus' completely separated constitutes thus an artificially separated bundle of muscle fibers. Thoma⁸ has also appreciated the fact that a muscle fasciculus is not a unit of structure. In serial cross sections of the gastrocnemius of the frog, in which, with the aid of the camera lucida, the outlines of the muscle fibers were sketched serially he noted single muscle fibers passing from one muscle fasciculus to another, concluding as follows: "Die einzelnen Muskelfaserbündel hängen somit vielfach durch Muskelfasern zusammen, welche bald mit dem einen, bald mit dem anderen Bündel sehr innig verbunden sind, und die ganze Muskelmasse bildet ein stark in die Länge gezogenes Netzwerk von Muskelfasern." The fasciculus above referred to as containing 26 fibers is to be considered in this light. In the figure as drawn, at each end 5 fibers begin with blunt ends showing by form, structure and staining that they are muscle fibers ending in tendon. Each of the fibers extends into the fasciculus for a distance which varies for the several fibers, becoming attenuated and finally terminates in a thread like filament having a thickness of 3 μ to 4 μ . It requires very

⁸ Thoma, R., Über die netzförmige Anordnung der quergestreiften Muskelfasern. Virchow's Archiv, vol. 191, 1908.

thorough maceration to enable one to separate completely these fine, intrafascicular terminations of the muscle fibers. The length of the muscle fibers having one tendon end at the end of the fasciculus, the other ending in an intrafascicular filamentous termination varies as follows; fiber a, 2.1 cm.; fiber b, 1.8 cm.; fiber c, 1.5 cm.; fiber d, 1.3 cm. The other fibers of this type sketched are intermediate in length between fibers a and d. The three spindle shaped intrafascicular fibers with both extremities attenuated and neither end reaching the tendon ends of the fasciculus measure as follows, fiber e, 2.1 cm.; fiber f, 1.9 cm.; and fiber g, 1.7 cm. The extent of overlapping of fibers beginning at the tendon end of the fasciculus and ending intrafascicular in fine, attenuated ends may be noted in this figure (2). Their exact relation cannot be readily seen in a completely teased preparation, with fibers separated and arranged on the slide. To gain their relationship actual teasing is necessary. While teasing the details of the arrangement of the muscle fibers becomes evident and it is observed that the fine filamentous intrafascicular ends are applied usually to the thicker portions of other fibers, usually not near a filamentous end of another fiber. The same is true of the ends of the spindle-shaped fibers reaching neither fascicular end. This figure (2) I regard as representative of the form and arrangement of the striated voluntary muscle fibers in the fasciculi of rabbit muscles having a fascicular length of from about 3 cm. to about 5 cm. Probably the same is true of voluntary muscle of other vertebrates, though my observations have not been extensive outside of rabbits and birds (rooster).

In muscle with longer fasciculi the length of the muscle fibers having blunt tendon ends and filamentous intrafascicular terminations varies more than indicated by the measurements above given, and the spindle shaped fibers with intrafascicular position may lie nearer one end or the other of the respective fasciculus or occupy a more middle position. This variation in the length of the muscle fibers I have indicated in figure 3, giving type fibers from a fasciculus having a length of somewhat over 4 cm., and taken from one of the thigh muscles. Un-

fortunately the specific muscle could not be determined after the maceration. This fasciculus was also completely teased and successfully mounted. It contains 37 fibers of which 8 are spindle shaped and have an intrafascicular position. In it one fiber extends from end to end, through the length of the fasciculus. The fibers could readily have been sketched in approximate relative position with reference to the ends of the fasciculus, but the resulting figure, at the magnification used, would have been too long to admit of publication in the pages of this Journal. The arrangement of the fibers, however, is not unlike that presented in figure 2. For figure 3, type fibers were selected. The single fiber extending from end to end could not be included by reason of its length. The fibers having a tendon end are arranged with reference to an imaginary line, at the bottom of the figure; the tendon end being brought to this line. Of certain of the fibers with tendon ends and intrafascicular filamentous terminations the actual lengths are as follows, fiber a, 2.9 cm.; b, 2.4 cm.; c, 1.92 cm.; d, 0.9 cm.; e, 1.4 cm.; f, 0.14 cm. The spindle shaped fibers sketched with both ends terminating intrafascicular with filamentous endings present the following measurements, fiber g. 2.9 cm.; h, 3.04 cm.; i and j, 2.9 cm. The single fiber extending through the entire fasciculus presents a length of almost 4.5 cm.

For rabbit muscle fasciculi having a length of more than 4.5 cm. to about 5 cm., so far as my observations go, there are no muscle fibers that extend the whole length of a respective fasciculus. In some of the longer fasciculi taken from the latissimus dorsi, the pectoralis major and the extensor cruris almost complete teasing was obtained. Many muscle fibers were completely isolated, though never all of the fibers of a given fasciculus. In some of the most successfully macerated fasciculi, their distal ends were slightly crushed during removal, so that not all of the fibers could be traced to their tendinous ends. For final teasing of these longer fasciculi, lantern slide covers answer the purpose of slides very well. In the longer fasciculi, having a length of 6 cm. to about 6.5 cm., in which many fibers were completely isolated, no fibers were found

reaching from end to end. Fibers with blunt tendon ends and filamentous intrafascicular terminations, these, severally of varying lengths, and spindle shaped fibers with intrafascicular position, with ends terminating in hair like processes, constituted the types of fibers isolated. In these longer fasciculi one end of certain of the spindle shaped fibers reaches nearly to one or the other tendinous end of the respective fasciculus while others of the spindle shaped fibers have a more nearly central position, with reference to the length of the fasciculus. In the longer and longest fasciculi teased, no muscle fibers having a length of more than about 3.5 cm. were observed.

Felix⁹ is quoted as having isolated striated muscle fibers approaching a length of about 12 cm. In his account stress is laid on the fact that in the macerating fluids used, acids mainly, the muscular tissue contracts by one-third to two-thirds of the original length. In his own material he sought to obviate this contraction by maintaining the original length through tension. I have noted the fact that in the method used, hydrochloric acid is injected into the living muscle while under extension, that during immersion in the hydrochloric acid and in the hematoxylin stain, a contraction of the muscle fasciculi to about two-thirds to one-half of their original length is obtained, but also that in the ammonia water fasciculi of muscle taken from the hematoxylin solution extend in length so as to approach very nearly their length in fresh muscle. Exact measurements I am unable to give since, obviously, it would be necessary to isolate at least small bundles of fasciculi from fresh muscle, and trace them through the various steps, making measurements at various stages. Of the longest fibers isolated by Felix, one from the gracilis of man measured 11.5 cm. and one from the sartorius of man 12.3 cm.; the latter fiber having a broken end. Division of fibers was not seldom found. A figure of a single fiber with branchings is reproduced natural size. This fiber in the figure measures approximately 12 cm. Concerning this fiber the text speaks as follows: "Die Faser theilt sich, lässt

⁹ Felix, W., Die länge der Muskelfaser bei dem Menschen und einigen Säugethieren. Festschrift, Albert von Kölliker, Englemann, Leipzig, 1887.

Spalträume erkennen, steht mit anderen Fasern in Verbindung, kurz um, das Bild wird durch vielfach abgehende Fasern ein so complicirtes, dass man ein Gewirr von mehreren Fasern vor sich zu haben glaubt, bis eine genaue mikroskopische Untersuchung ihre Zusammengehörigkeit nachweist." Felix teased unstained tissue. I have not teased human muscle. However, the figure presented by Felix is not unfamiliar to me. In incompletely macerated tissue such 'fibers' are now and then obtained. However, they are interpreted by me as representing an incompletely teased fiber complex. The fine hair like intrafascicular ends of muscle fibers are so closely applied to the sides of other fibers that the cross diameter of the thicker fiber is scarcely increased. Such a misinterpretation, I can conceive, may readily be made in incompletely macerated and teased muscle tissue. Felix gives data concerning the length of muscle fibers in the rabbit, a tissue with which I am familiar. This observer isolated fibers from the pectoralis, sartorius, latissimus dorsi and extensor cruris of the rabbit. His own words concerning them read as follows: "Hier waren fast sämtliche Fasern mindestens 5 cm. lang, doch waren unter 6 cm. nur wenige zu erzielen. Die meisten Fasern schwankten zwischen 6.0 und 7.5 cm. Die Fasern zeichneten sich sämtlich durch ihre Stärke aus. Die längste Faser isolierte ich aus dem extensor cruris, der am Thiere selbst nur 8 cm. mass, von 8 cm. Länge. Die Dicke war ungemein schwankend, dickere und dünnere Stellen wechselten ab, die dünnste Stelle mass nur 0.0109 mm., während dickere Stellen 0.111 mm. gemessen wurden. Offenbar sind hier verschiedene Wirkungen der Salpetersäure zur Geltung gekommen. Theilung konnte ich häufig beobachten."

An analysis of this statement from Felix in the light of my own investigations leads me to conclude that this observer did not obtain completely teased muscle fibers. Many hundreds of muscle fibers of the rabbit have been completely isolated and in no instance have I observed branching of fibers. Often have I seen apparent branching, but on careful teasing such structures have been separated into several fibers. The variation in thickness of the long fibers referred to in the above quotation, I be-

lieve, is explained by a linking in chain of several fibers. Even granting that the fasciculi teased by me after a stay in ammonia water, some attaining a length of about 6.5 cm., had not attained their full, original length, the difference in the length of muscle fibers of the rabbit teased by Felix and by myself is not accounted for. Felix found few attenuated ends of fibers with intrafascicular terminations, while, as my own figures show, these are numerous. In the light of these studies I am inclined to regard the measurements of the length of striated voluntary muscle fibers as given by Felix as inaccurate and as made on incompletely teased muscle tissue, and to regard the figures given by earlier observers as more accurate. These, to quote freely from Felix, are for the medium length of muscle fibers of man 2 cm. to 3.5 cm., Krause giving as the longest of the fibers of the sartorius 4 cm.

Striated voluntary muscle fibers of other mammals and other vertebrates have thus far been only incidentally teased by me. Bardeen's² figure 4, b, gives a flat band of fibers dissected from the external oblique of a dog, having a length of approximately 15 cm. (figure one-half natural size) with figures of completely isolated fibers; spindle shaped fibers having a length of approximately 8 cm. and fibers with blunt tendon ends and attenuated intrafascicular terminations, varying in length from approximately 4 cm. to 6 cm. The general shape of these fibers appears to me as correctly drawn. Since I have not teased muscle fasciculi of the dog I am unable to verify the accuracy of the measurements given. For the dogs muscle fibers Felix gives 3 cm. to 4.5 cm. as common measurements and 5.5 cm. to 6.5 cm. as long fibers.

Opportunity presented itself to tease muscle fibers of an adult rooster (*Gallus domestica*), injected with hydrochloric acid for other purposes. In one specimen, the thigh muscles were well macerated. In figure 4 are shown four completely teased spindle shaped fibers taken from these muscles. These fibers, some of which are among the longest completely teased, present the following measurements: fiber a, 3.2 cm.; b, 3 cm.; c, 3.2 cm. and d, 2 cm. Several spindle shaped fibers with intra-



Fig. 4 Spindle shaped muscle fibers teased from the thigh muscles of an adult rooster (*Gallus domestica*). Actual length of fibers, a, 3.2 cm.; b, 3 cm.; c, 3.2 cm.; d, 2 cm. $\times 5$.

fascicular position, with undoubted branching were observed. The division extended to about the middle of the respective fibers, the two parts terminated in attenuated, hair like fibers. Muscle fibers with blunt tendon ends and filamentous intra-fascicular terminations were also observed.

It is the purpose, as opportunity presents, to include in this study fibers from different types of muscles from the different classes of vertebrates and to extend the investigation so as to include several different mammals with types of muscle from each.

Schiefferdecker¹⁰ and certain of his pupils have spent infinite pains in determining, among other things, the relative thickness of muscle fibers. The thickness and form of muscle fibers these workers have determined largely in cross sections of various muscles. Each muscle is said to be composed of muscle fibers having specific size and form (cross section) with specific arrangement of connective tissue and elastic fibers. It is recognized that in each muscle, muscle fibers of varying sizes are found. In many muscles this difference in size of fibers is said to be considerable, in others less so. This difference in size of fibers may be ascribed, according to Schiefferdecker, to two possibilities: 1, the muscle may be composed of fibers which in reality differ in size; 2, the smaller and smallest cross cut fibers of a given cross section may represent cross sections of the ends of fibers terminating in the muscle. In considering the structure of muscle, he adds, the second possibility plays only an unimportant rôle, and only as concerns the smallest fibers. The fibers sketched in figures 2 and 3 may serve to show that such contention is difficult to support in the light of this work. Except for muscles in which the fibers of the respective fasciculi extend from end to end, or in which the majority of the fibers do this, the variation in the size of the fibers in a given cross section is largely dependent on the fact that many of the fibers of a given fasciculus do terminate intrafascicularly. In order to make the numerous measurements of Schiefferdecker and his pupils of real value, or of similar investigations, it would be necessary to ascertain by means of teasing and complete isola-

¹⁰ Schiefferdecker, P., *Muskeln und Muskelkerne*. Barth, Leipzig, 1909.

tion of fibers, the arrangement of the fibers in the fasciculi of muscles, the fibers of which are measured in cross sections.

MacCallum's¹¹ investigations led him to conclude, as a result of counting the fibers of the sartorius muscle in man at various ages that the muscle fibers cease to multiply in the fetuses from 13 cm. to 17 cm. in length, and that after that period muscles increase in size by increase in size of individual fibers. This statement, it would seem to me, needs verification and could only be verified by study of muscles in which all of the fibers of the fasciculi extended from end to end or by very careful and painstaking teasing, of fasciculi, covering the several periods in which the muscle fibers are counted.

Myofibrils are usually regarded as extending from end to end in a given muscle fiber. In muscle fibers having filamentous intrafascicular terminations, and this includes the majority in the longer fasciculi, this is obviously not the case. Concerning the relations of the ends of myofibrils not reaching the ends of the respective muscle fibers, my teased preparations give no evidence. The festooning of the sarcolemma, described by certain authors, may perhaps be brought in relation with the ends of myofibrils which do not extend the entire length of the muscle fiber.

In this communication the expression "completely teased and isolated muscle fibers" has been repeatedly used. Therefore it will no doubt seem paradoxical, for me to express in this concluding paragraph, even tentatively, the view that striated voluntary muscle is syncytial in character.

From the arrangement of muscle fibers in the fasciculi of striated voluntary muscle; from the fact that muscle fasciculi are not units of structure; from the further fact that in teasing muscle fibers there are always found points of contact where the fibers are ultimately separated with great difficulty, I am led to tentatively express the view that striated voluntary muscle tissue presents syncytial character even in its fully developed state, as does involuntary muscle and cardiac muscle, though

¹¹ MacCallum, J. B., On the histogenesis of striated muscle fibers and the growth of the human sartorius muscle. Johns Hopkins Bull., 1898.

not to the same degree as the last named. This question cannot be finally decided by teasing. It is not my purpose at the present time to enter upon the mooted question of the histogenesis of voluntary muscle tissue, nor to consider the extensive literature involved. The problem of the syncytial character of voluntary muscle is one of histogenesis. Embryological evidence at hand indicates that the histogenesis of voluntary muscle lends support to the view that striated voluntary muscle is syncytial in origin. Material is being collected to determine this question if possible. One of Schiefferdecker's¹⁰ general conclusions reads as follows: "Muskelnetze fanden sich in den untersuchten Muskeln so vielfach, dass man sie wohl als eine allgemein verbreitete Erscheinung ansehen kann." Thoma⁹ finds frequent anastomoses between fibers. Reference, however, is not had to anastomoses between fibers such as described by Thoma. This observer finds intimate contact between adjacent fibers, so that for a distance only a single layer of sarcolemma appears to separate them. Myofibrils are not thought to pass from one fiber to another. It has seemed to me that this may be verified in teased preparations. Now and then two fibers adhere together, for a short distance, so closely, that separation, even in well macerated tissue, is impossible; this very generally in thicker portions of fibers. Involuntary muscle, if successfully macerated in potassium hydrate or by the hydrochloric acid method here detailed is readily teased so as to present spindle shaped cells, although as shown by McGill¹² this muscle develops from mesenchyme, retaining its syncytial character. The mere arrangement of striated, voluntary muscle fibers in a fasciculus possessing fibers with attenuated intrafascicular terminations, is such as to suggest the syncytial character of this tissue. In partially teased, though well macerated tissue, a mesh work of fibers, with long meshes is now and then evident. It is usually possible to tease the fibers having intrafascicular termination, quite readily, so far as concerns the thicker portions of these fibers and to isolate them to near their thread like terminations

¹² McGill, Caroline, The histogenesis of smooth muscle in the alimentary and respiratory tract of the pig. *Monatschrift Anat. u. Phys.*, vol. 24, 1907.

on other fibers. Near their intrafascicular ends they adhere very tenaciously to adjacent fibers. In ammonia water the macerated and stained fibers become quite pliable and present an elasticity and a tensile strength which is often surprising. Yet, often the finer ends are broken before they can be detached from adjacent fibers. It is evident that the relations of the intrafascicular ends of muscle fibers to adjacent fibers is different at their attenuated terminations than in course. Their exact relation I am unable to determine in teased preparations, though even the finest ends often present the appearance of a torn sarcolemma which does not extend to the extreme tip. I am unable to state whether the myofibrils extend from the attenuated ends to fibers on which they appear to end. In a number of preparations of rabbit embryos of the tenth day, cut serially in the sagittal plane, sections having a thickness of $2\ \mu$ and $3\ \mu$, stained in iron-lac-hematoxylin, the syncytial character of the cells from which the voluntary muscle tissue is developing is evident. Conclusive preparations, from embryos varying in ages, have thus far not been obtained. This question shall form the subject of a further study now under way. It may be recalled here that Godlewski¹³ considers striated muscle as presenting a syncytial character, basing his deductions on a study of the histogenesis of skeletal and heart muscle.

It is impossible at the present time to do more than suggest that striated voluntary muscle, like involuntary and cardiac muscle, presents a syncytial character, evidence of which is seen in its full development.

¹³ Godlewski, E., Die Entwicklung des Skelet- und Herzmuskelgewebes der Säugethiere. Arch. f. Mik. Anat., vol. 60, 1902.

A NOTE ON THE STRUCTURE OF THE ELASTICA INTERNA OF ARTERIES

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ONE FIGURE

A comparison of a number of texts, descriptive of the structure of the elastic intima—the fenestrated membrane of Henle—of arteries, reveals the fact that the views concerning the structure of this layer are by no means unanimous. Schäfer¹ speaks of the elastic intima as follows: “The elastic tissue is represented by one distinct lamina, which is separated from the endothelium by the subendothelial layer. It is, on its outside, in direct contact with the non-striped muscle of the middle layer.” The ‘internal elastic lamina’ is spoken of as membranous in character, the membrane is not, however, a continuous one, but is perforated by apertures. In figure 517, of Schäfer’s text, is shown a portion of the fenestrated membrane from the femoral artery as figured by Henle. Mall² in his study of connective tissue fibrils states that “elastic fibers are composed of two distinct substances—the interior, which stains intensely with magenta, and the membrane, which does not.” A study of the membrane of Henle, isolated by boiling in acetic acid or KOH and then stained with magenta or picocarmine leads him to conclude that “The Henle’s fenestrated membrane is therefore composed of three layers—an upper and a lower transparent membrane in which there are no openings, and which is identical with the membrane of elastic fibers; and a middle layer which stains with magenta, and is identical with the interior of elastic

¹ Schäfer, E. A. Text book of microscopic anatomy. Longmans, Green, and Co., 1912, p. 332.

² Mall, F. P. Reticulated tissue, and its relation to connective fibrils. Johns Hopkins Hospital Reports, vol. 1.

fibers. This central portion contains the openings." V. Ebner's³ description of the elastic intima concludes as follows, "Uebrigens erscheint dieselbe fast immer als eine sogenannte gefensterte Haut mit verschiedenen deutlich ausgeprägten, netzförmigen Fasern und meist kleinen länglichen Oeffnungen, seltener als ein wirkliches, aber sehr dichtes Netz vorzüglich langsverlaufender elastischer Fasern mit engen, länglichen Spalten, und stimmt in ihrem chemisches Verhalten vollkommen mit den elastischen Häuten der Media grosser Arterien überein." Triepel⁴ in his account of the elastic tissue in the walls of intracranial arteries considers the elastic intima as a fenestrated membrane, stating that in the smaller arteries the fenestra are so near together that only a felt-work of elastic fibers remains, so that in cross sections a row of adjacent points is observed instead of a membrane. His figure 4 shows this clearly. Schöppler,⁵ who studied the finer structure of the brain arteries of several mammals, gives especial consideration to the internal elastic membrane, and gives emphasis to closely arranged longitudinal ledges, which have a course parallel to the long axis of the vessels. He recognizes a fibrillar structure in the elastic intima as expressed in these words, "Vielfach zeigt sich auch, dass die Membrana flava interna keine homogene Platte ist, sondern wie die Betrachtung von Schrägschnitten bei 1000facher Vergrösserung lehrt, aus, sehr feinen elastischen Fäserchen besteht. Die erwähnten Leisten werden durch Ausbildung stärkerer nach dem Lumen vorspringender Fasern bedingt." His figure 6, which shows an oblique longitudinal section of a basilar artery presents the fibrillar character of the elastic intima clearly. Dürck⁶ records observations made on connective tissues studied by means of Weigert's iron-hematoxylin myelin sheath staining method. In tissues fixed in formalin and Müller's fluid or in formalin,

³ v. Ebner, Victor, *Köl liker's Handbuch der Gewebelehre des Menschen*, Dritter Band, Zweite Hälfte. Engelmann, Leipzig, 1902, p. 643.

⁴ Triepel, H. *Das elastische Gewebe in der Wand der Arterien der Schädelhöhle*. Anat. Hefte, vol. 7, 1897.

⁵ Schöppler, H. *Ueber die feinere Struktur der Hirnarterien einiger Säugtiere*. Anat. Hefte, vol. 15, 1900.

⁶ Dürck, H. *Ueber eine neue Art von Fasern im Bindegewebe und in der Blutgefässwand*. Virchow's Archiv, vol. 189, 1907.

mordanted in a copper salt and stained in iron-hematoxylin, following the Weigert method for staining myelin sheaths, certain connective tissue fibrils were stained blue-black. Certain of these differentially stained fibrils were regarded by Dürk as a special type of connective tissue fibrils, others, as yellow elastic fibers. This method as used by this observer, gave, in successful preparations, unusually distinct staining of the elastic intima of vessels. His words read as follows, "Untersucht man zunächst kleine Arterien auf dem Längsschnitt oder auf Schrägschnitten, welche das Rohr in langer Ausdehnung treffen, so erkennt man an den durch die Intima fallenden Schnitten, dass die Elastica interna hier nicht durch zirkuläre Fasern, Faserbündel oder Lamellen dargestellt wird, wie man dies gewöhnlich abgebildet und beschrieben findet, sondern unmittelbar über dem Endothelrohr liegt wie eine Basthülle unter einer Baumrinde eine einfache Schicht von straffen Längsfasern, welche unter sich allerdings durch kurze quere Zwischenstücke verbunden sind und so ein Netz mit sehr langgestreckten und längs verlaufenden Maschen darstellen." In cross-sections such fibers appear as points.

The method used in staining the sections on which this study was based and from one of which the figure accompanying this note was drawn, was presented by Dr. De Witt⁷ at the Wisconsin meeting of the American Association of Anatomists in 1907. This differential elastic tissue staining method consists of a modification of Weigert's⁸ iron-hematoxylin van Gieson method. According to Weigert's method two stock solutions are prepared.

Solution I

Hematoxylin crystals.....	1 gram
Alcohol, 96 per cent.....	100 cc.

Solution II

Liquor ferri sesquichlorati (U. S. P.).....	cc.
Hydrochloric acid (sp. gr. 1.20).....	40
Aqua dist.....	7
	959

⁷ DeWitt, Lydia M. Abstracts of papers presented at the 22nd Session Amer. Ass. Anat. Anat. Record, vol. 1, p. 74.

⁸ Weigert, K. Eine kleine Verbesserung der Hämatoxylin-van Gieson-Methode. Zeitsch. f. wissensch. Mik., vol. 21, 1904.

Solutions I and II are mixed in equal proportions just before using. Differentiation is obtained by means of van Gieson's picric acid fuchsin solution, prepared after Weigert as follows:

Picric acid, saturated aqueous solution.....	cc. 100
Acid Fuchsin (Weigert), 1 per cent aq. sol.....	10

In the method as used for elastic tissue staining, stock solutions I and II are mixed in proportion of 3 to 4 parts of solution I to one part of solution II. The sections are stained several hours and after rinsing in distilled water differentiated in van Gieson's picric acid, acid fuchsin solution, prepared as above indicated. The differentiation is controlled from time to time under the microscope. The method is simple and can be used on celloidin sections or paraffin sections fixed to the slide. The yellow elastic fibers are stained blue-black, the collagenous tissue is stained brick-red to pink, depending on the degree of differentiation and the thickness of the sections. The method is not unlike that used by Dürck, although the differentiation by means of the van Gieson picric acid, acid fuchsin solution has the advantage of counterstaining the collagenous tissue. This differential elastic tissue staining method has been extensively used in the preparation of sections for classes and is recommended as simpler than other differential elastic tissue stains.

Numerous sections of arteries varying in size from arterioles with two or three layers of muscle cells to arteries of about 2.5 mm. in diameter, cut in pieces of tissue fixed in formalin, formalin and Müller's fluid, and picro-nitric solution, embedded in paraffin, and sections fixed to slides, were stained after the above mentioned iron-hematoxylin and picric acid, acid fuchsin method. The differentiation in picric acid, acid fuchsin was carried in most sections to an extreme degree, so that only the yellow elastic tissue retained any of the blue-black coloring. Usually four to six sections were fixed to one slide, the sections approximating 5μ in thickness. They were cut on the sliding microtome, thus varied a little in thickness and gave slightly varying degrees of differentiation. The larger and largest arteries were, owing to want of suitable material, not included in this special

study, though previous incidental study of such vessels leads me to believe that the elastic intima, where present as such, is in general character like that of the smaller vessels. In no instance were the arteries especially studied removed from the surrounding connective tissue, so that the staining of the elastic tissue in the perivascular areolar tissue served as a control for the staining of the elastic tissue in the arterial walls.

In all of the successfully stained preparations and in arteries varying in size from the smaller to the larger ones studied, the elastic intima appears, when successfully differentiated, as a

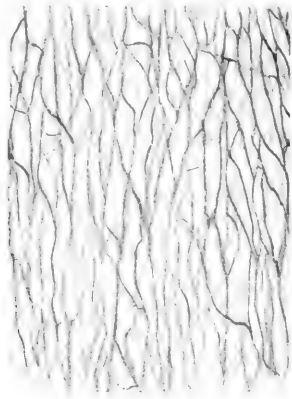


Fig. 1 Elastic intima of deep plantar artery, human. Stained in iron-hematoxylin and van Gieson's acid fuchsin, picric acid solution. $\times 600$.

network of elastic fibers, the larger fibers of the network having in the main a direction which is parallel to the long axis of the respective vessel. A well stained and well differentiated longitudinal or longitudinal oblique section of an artery including the elastic intima, appears not unlike a successfully teased preparation of yellow elastic tissue from the ligamentum nuchae.

In figure 1 is presented a drawing of a portion of the elastic intima of one of the larger deep plantar arteries of a human foot. During fixation the artery had collapsed in such a way that on one side, for a distance, its wall was nearly in a plane. Several sections of a series thus included long stretches of the elastic intima. In this figure only the elastic tissue, which is

stained deeply blue-black, is reproduced as drawn with the aid of the camera lucida, using a $\frac{1}{12}$ inch oil immersion objective and a No. 4 Zeiss compensation ocular with paper at table level. The network character of the coarser elastic fibers with frequent anastomoses and numerous cross-bridges is faithfully reproduced. It was not possible to draw accurately all of the finest fibrils throughout their entire extent. However, the figure as a whole gives a correct impression of the appearance presented by the section. At both ends (above and below the figure), the intima leaves the plane of section and the elastic fibers, shown as a network in the figure, appear as cross cut or obliquely cut fibers. In numerous other sections of vessels of varying sizes, longitudinally or obliquely cut, including the elastic intima, similar appearances are found. The character of the network varies but slightly, dependent on the degree of extension or distension of the respective vessel. Oblique sections approaching cross sections of vessels are especially instructive. In such sections a side view of the elastic network of the elastic intima with end view of the fibers as seen in cross-cut, is obtained by moving the micrometer screw of the microscope. In cross sections of vessels, in place of the usual line representing the elastic intima as seen after the usual staining, there is observed a row of deeply stained blue-black dots, varying in size with the size of the vessel, with here and there a longer or shorter blue-black dash where a cross anastomosis between fibers is included in the section.

Sections of areolar connective tissue, differentially stained for elastic tissue by means of the iron hematoxylin picric acid, acid fuchsin method, present no evidence of an 'outer membrane' for elastic fibers as described by Mall. However, the existence of such a membrane is in no sense denied, since a slight tinging with picric acid would not be evident against the deep blue-black stain of what is probably the 'inner substance,' stained readily in magenta. In certain of the longitudinal sections of vessels including the elastic intima, as for instance in the section from which the figure here presented was drawn, a delicate grey-blue color overlies the elastic network. This is represented in the figure by a light wash of neutral tint. If

this be expressive of structure it reveals a homogeneous structure and may possibly indicate the presence of a homogeneous membrane. Such a membrane, however, I have not detected in cross sections of vessels.

From this study of the elastic intima of arteries the conclusion seems warranted that the stainable substance of this layer consists of a network of yellow elastic fibers, with coarser fibers having in the main a course which is parallel to the long axis of the respective vessel, these fibers presenting frequent anastomoses and cross bridges, and with numerous finer fibrils which pervade the network. Here and there certain of the fibers of the elastic intima may in cross or oblique sections be traced in anastomosis with elastic fibers of the media. It would thus appear desirable to discard the term 'fenestrated membrane,' since this term does not express the structure of this layer. Of previous descriptions, that given by Dürk appears to me the most nearly conforming with observed facts.

A NOTE ON THE MORPHOLOGY OF THE SEMINIFEROUS TUBULES OF BIRDS

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ONE FIGURE

In the course of a study of the renal tubules of birds, by means of the maceration method devised by Huber,¹ in which full grown roosters (*Gallus domestica*) were used as material, the injection of the 75 per cent solution of hydrochloric acid was through the aorta central to the branches supplying the kidneys and sex glands. In a number of the cases the testes were found to be well injected and were removed and placed in 75 per cent hydrochloric acid with a view of obtaining thorough maceration preparatory to teasing. Following the method as described, the macerated pieces were washed thoroughly in distilled water, stained in hemalum, softened and cleared in 0.25 per cent to 0.5 per cent ammonia water, in which they were teased.

Even during the preliminary teasing of the larger pieces it was noted that the testis of the rooster was not separable into lobular masses, as is the case in mammalian testes, so that it was found impossible to isolate structural units with which the final teasing could be carried out.

Huber and Curtis² found that in the mammalian testis the seminiferous tubules presented no blind ends, diverticuli or nodular enlargements but were arranged in the form of an arch or a variable number of linked arches, all of the ends terminating

¹ Huber, G. Carl. A method for isolating the renal tubules of mammalia. *Anat. Rec.*, vol. 5, 1911.

² Huber, G. Carl, and Curtis, George Morris. The morphology of the seminiferous tubules of mammalia. *Anat. Rec.*, vol. 7, 1913.

in tubuli recti attached to the rete testis. Wax reconstructions made by Curtis confirm the observations made on teased preparations. Repeated teasings have convinced me that in the adult bird no such arrangement of tubules pertains, but that the seminiferous tubules of the bird are arranged in the form of a network, presenting a varying number of anastomoses found at different levels in the gland substance. For this reason the teasing of these tubules is exceedingly difficult in that it is impossible without breaking or tearing tubules to separate favorable pieces preparatory to final teasing. All of my teased preparations present an endless net, with broken tubular ends as a boundary.

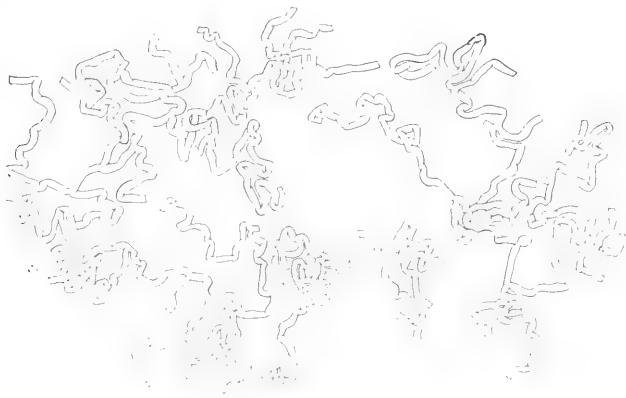


Fig. 1 Teased preparation showing a portion of the tubular system of the testis of the bird (*Gallus domestica*). $\times 5$.

In the accompanying figure is presented one of the most successful preparations obtained. The figure was traced and sketched with the aid of the camera lucida at a magnification of 50 diameters, reduced to a magnification of 5 diameters in the reproduction. The portion of the tubular net reproduced presents in all some forty broken ends, and at least three closed rings. Such closed rings I have found in nearly all of my preparations. Their complete separation requires great care and patience since uniting tubular portions are very easily broken. The clearness of the figure, it is thought, obviates the necessity

of extended description of the character of the network formed by the seminiferous tubules of birds. The figure, however, should be studied with the understanding that in the mount from which the figure was drawn the teased tubules were spread out as much as possible. The figure, therefore, does not give spatial relations of the tubules. In the gland, as is well known, these tubules form compact coils, evident somewhat from the extended kinks and bends seen in the figure.

In a rather careful search of the literature I have been unable to find any description of the form of the seminiferous tubule of birds. This note would thus seem justified. However, the observations here recorded would seem to me to have a bearing on previous work emanating from this and other laboratories, relating to the form of the seminiferous tubules of mammals. The results here recorded seem to me to confirm the observations made on teased preparations of the seminiferous tubules of mammals. The fact that complex anastomoses resulting in closed ring structures have been teased in the bird's testis argues for the possibility of teasing such structures in the adult mammalian testis, did they exist. Bremer,³ as a result of careful wax reconstructions of the tubular system of the human testis, working on embryonic and fetal tissue, the oldest stage studied being that of a human fetus of seven months, reached the conclusion that

The testis cords, growing from the germinal epithelium of the genital ridge, form a network with three sets of anastomosing branches. After completion, this network breaks down partially, leaving certain cords as persistent stems. The tubules of the adult show, in their course, connection, and position in the testis, traces of this network. Testis tubules may be single, ending blindly, may branch, or may anastomose.

In the adult mammalian testis tubules, completely teased, no blind endings, buds, nor ring formations were observed, while in the teased preparations of the seminiferous tubules of birds, the remains of the network of tubules as observed in the embryonic and fetal stages and well figured by Bremer, may be

³ Bremer, John Lewis. The morphology of the tubules of the human testis and epididymis. *Amer. Jour. Anat.*, vol. 11, 1911.

noted. In a cryptorchid of the rabbit, as described by Huber and Curtis, extended anastomoses of testis tubules were observed in two regions of the tubule complex, and in two regions, near the periphery of the gland, tubules were joined so as to form two folded rings. The preparations from the cryptorchid of the rabbit present appearances not unlike those shown in teased preparations of the seminiferous tubules of the bird. The presence of the remains of the embryonic network of the seminiferous tubules in the cryptorchid of the rabbit and in the bird's testis, postulates a relatively late, complete morphogenesis of the seminiferous tubules of the mammal. Phylogeny and ontogeny indicate this. In the light of these observations I am of the opinion that Bremer's careful study of the morphology of the seminiferous tubules of the human testis are of value as concerns embryonic and late fetal stages, but may not be transmitted to the adult gland in that in the oldest stage studied, a human fetus of the seventh month, the seminiferous tubules, in all probability, had not completed their morphogenesis. The question is one deserving further study and will form the subject of a future, more comprehensive communication, based on especially prepared and 'timed' material from the rabbit. This form is chosen since the morphology of the seminiferous tubule of the adult rabbit has received special consideration in this laboratory, both by means of teased preparations and reconstructions.

THE HISTOLOGY OF BLOOD AND LYMPHATIC VESSELS DURING THE PASSAGE OF FOREIGN FLUIDS THROUGH THEIR WALLS

II. STUDIES ON ABSORPTION FROM SEROUS CAVITIES

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It is at once evident to any one who studies the enormous literature which has been published during the last century, on the absorption into the body of foreign material from the serous cavities, that the problems presented to the present day investigators in this field may be roughly grouped under four heads, as follows: 1) the localization of absorbing surfaces; 2), the definite establishment of the channels of removal; 3) the determination of the forces concerned in the passage of matter through the walls of the serous cavities, and its entrance into and exit from the vessels which distribute it about the body for storage and digestion, or for destruction and excretion; and 4) the location and identification of the organs concerned in the storage or destruction of substances or fluids absorbed.

Up to the present time not even the questions which are included under the first and fourth of these heads can be said to have been sufficiently answered; the second has been settled definitely only for a single area and with a limited number of substances, and about the third practically nothing is known.

Although a vast amount of work has been done on the localization of absorbing surfaces and their related organs of storage and excretion, until quite recently but little was known regarding them; and this is so in spite of their immense clinical importance in connection with the postural treatment of the various serousitides.

It is owing to the work of MacCallum (1), who made a careful and productive study of the removal of foreign granules from

the peritoneal cavity through the lymphatic lacunae of the diaphragm, that practically all who have made a study of serous absorption accept it as a fact that a great deal of any solution or foreign body introduced into the peritoneal cavity passes through the peritoneal surface of the diaphragm, and enters the system of the experimental animal or the patient via its vessels.

The position of other foci of drainage has remained unknown, and the tendency to deny or ignore their existence has been and is very strong.

A few observers have suggested the broad surface of the greater omentum as a possible agent in the removal of foreign fluid from the peritoneum, but their assertions have rested upon probability rather than definite proof.

Rubin (2), who attacked the problem from an experimental basis, showed, however, that less fluid was absorbed from the peritoneal cavities of animals whose omenta he had amputated, than from the peritoneal cavities of normal controls; and Crouse (3) after careful study concluded that the omentum is an important factor in the mechanism of peritoneal drainage, and hypothesizes a protective lymphatic drainage to account for the phenomena which he has observed. The authors (4) have been able to show experimentally that beyond doubt the omentum is a very efficient agent in the drainage of the peritoneal cavity. By drawing the omentum out of the animal's body through a mid-line incision, and keeping it immersed in a fluid medium under physiological conditions, it was possible not only to isolate the organ and to prevent the experimental fluid from reaching other surfaces, but also to eliminate any influence on absorption which might be exerted by the increased abdominal tension which follows the intra-peritoneal injection of large amounts of the fluid. In spite of conditions which might be supposed to make for secretion rather than absorption, we found that a large amount of the fluid in which these omenta were immersed passed into the omental vessels, and reaching the general circulation, was carried at once by the blood stream to the organs of excretion, from which the test fluid could then be recovered.

As to the second question, the establishment of the vascular system concerned in the drainage, for example, of the peritoneum, Meltzer (5), Muscatello (6), and others, held that drainage is accomplished through the lymphatics, while Heidenhain (7), Cohnstein (8), Dandy and Rowntree (9), and others, have shown that much of the fluid absorbed from the peritoneal cavity leaves it through the blood vessels. We have never been able to demonstrate the presence of lymphatics in the omental tissue of the adult cat, and Ranvier (10) claimed that while there are lymphatics in abundance in the omenta of young kittens many of them are obliterated by degenerative changes at the age of three months. If lymphatics exist in the cat's omentum they must necessarily drain in the same direction as those of the gastric system; that is, an omental lymphatic stream, if such a thing exists, must eventually become tributary to the lymph content of the thoracic duct.

In the experiments mentioned above with the omentum of the cat, the influence of lymphatic vessels was entirely eliminated in many of our experiments by the ligation of the duct. Hence we were able to prove not only that the omentum furnishes a surface where absorption takes place, but, by varying the fluid in which the omenta were immersed, we have shown that the removal of molecular solutions and colloidal solutions and of fine particulate matter in true suspensions may be accomplished through the blood vascular system to a large extent; though we do not by any means deny the probability of drainage through the lymphatic channels in the localities where these vessels exist. But when any attempt is made to ascertain, through the medium of existing literature, the forces concerned in the absorption of foreign matter from serous surfaces one enters at once into a region of guess and hazard, where only a few isolated facts exist as a guide to certain knowledge. We have only just ceased to argue for and against the presence of preformed 'stomata' and 'stigmata,' and to indulge in surmises as to their physiological significance. Students of the physiology of absorption are still discussing whether absorbed material passes through or between the lining endothelium of blood and lymphatic vessels and the

mesothelial cells of serous cavities. In our own experiments we found that a great deal of fluid may enter the blood stream when the influence of intra-abdominal pressure is removed; and, since our fluids were isotonic with the blood serum of the experimental animal, osmosis as it is generally understood, could have had only a negligible amount of influence upon the phenomena observed. Indeed if osmotic pressure had any influence at all, it would seem that it would have been exerted against rather than for the passage of the experimental fluid into the blood vessels, since even the small amount of fluid lost from the solution by evaporation from exposure to the air, must have changed an originally isotonic to a slightly hypertonic fluid, to which one might expect water to pass from the serum through the vascular wall. If such a passage occurred it in no way interfered with the imbibition of the experimental solution. We know very little about the part played by fluid pressure, the movement of the blood and lymph in their respective vessels, or the influence on serous absorption of the movement of contractile somatic organs, like the diaphragm, or the contraction of the musculature of the vessels themselves. We cannot say what chemical changes accompany or influence the transport of material from cavity to vessel; or whether the cytoplasm of the cells of the serous cavities, or of the blood and lymphatic vessels play any part in the transmission of matter through the vascular or serous walls. And does a disturbed balance of intra- and extra-cellular equilibrium militate for or against absorption? We do not know.

It will readily be seen that the examination of histological preparations made from the omentum during active drainage, may be of great value in strengthening the positive evidence for absorption through the blood vessels, and in aiding us to understand the mechanism of the removal of foreign matter through the vascular wall.

With this end in view, sections have been made and studied of omenta which, up to the time of fixation, had been exposed to and were absorbing all sorts of material from true solutions to mechanical suspensions. A report of the findings in this material is the purpose of the present paper.

By far the most valuable preparations were yielded by omental tissue which had been absorbing an isotonic solution of potassium ferrocyanide and iron ammonium citrate, and which was fixed immediately upon removal from that fluid in hydrochloric acid formalin with a resulting precipitation of prussian blue—the method used by Weed (11) to study the drainage of the cerebro spinal fluid.

An omentum so treated appears in gross to be stained a uniform pale blue except for the fat, and is patterned by an irregular network of an intense dark blue color. It is only necessary to examine the spread preparations with a binocular microscope to be convinced that the network is made up of the omental blood vessels whose lumina are filled with precipitated prussian blue; the picture is strikingly suggestive of a complete blood vascular injection of the omentum with a somewhat dilute prussian blue gelatine mass, and, in fact, we are dealing with much the same thing, since the coagulation of the colloidal proteids during fixation of the blood serum causes the same comminution of the nascent dye stuff which follows its precipitation in peptizing gelatin. In contrast to the general tissue which fills the meshes of the vascular net and which is very pale blue, or uncolored, a wide deeply stained zone of thickly precipitated dye surrounds each vessel.

The capillaries are all filled with prussian blue, even those supplying the perivascular fat being crowded with the dye and the capillary knots or glomeruli which form the support of many of the taïches laiteuse are completely injected. Here and there a capillary may be seen empty or nearly so, perhaps because contraction of its walls during fixation forced the absorbed dye from its lumen.

Of the larger vessels all have a greater or less amount of dye precipitate within the lumen. All are completely filled, but in some the blue color is perceptibly paler than in others. In general the arteries show much less absorption than the corresponding vein, but the depth of the color may not be the same throughout the length of a given vessel. There are often light and dark blue areas present. These preparations show that ac-

tive absorption is going on through the walls of the arteries as well as through the veins, even arteries with thick muscular walls taking part in the general process as will be shown below:

The larger vessels are paler than the smaller, probably because less fluid is taken in through their walls. In other words as the vascular size increases there is a gradually decreasing concentration of the intra-vascular dye solution, the significance of which will be discussed below.

From the point where the vessels begin to be surrounded by a perivascular sheath of fat the pallor of the precipitated dye in the vascular lumen markedly increases, evidently because the advent of the perivascular fat is accompanied by an increased thickness of the vascular wall and a diminished absorption. That the fat itself can have no effect in the decrease is shown by the intense color of the injection mass in the capillaries supplying the fat; and examination of sections shows that the ferrocyanide solution penetrates easily between the fat cells themselves.

Sections of the same material confirm the evidence of total spread preparations. The capillaries, even those imbedded in and supplying the fat, are distended with the blue color, and the veins are full of blue precipitate of varying depth of color. It is however not so easy to see the blue color in the larger arteries in sections.

It is possible even in thin sections to distinguish the deep blue perivascular area described above, and to trace its existence to precipitated dye in the intrafibrillar tissue spaces. Individual fat cells are outlined by dye precipitated from the fluid which has worked its way between the cells that it has never penetrated. Coarse precipitates of dye may be seen along the surface of the omentum and adherent to the surfaces of the elastic fibres. In some places the tissue is diffusely stained, and throughout the omentum, cells, probably of the clasmatocyte type are found, like those described by Weed in the meninges, whose cytoplasm is filled with fine granules of prussian blue, their nuclei however remaining uncolored. This intracellular precipitate is the result of imbibition of fluid by the

connective tissue cells, an adsorption phenomenon of the same nature as the drinking in of solutions of high molecular vital dye stuffs which is responsible for the diffuse cytoplasmic coloration seen early in a course of staining.

The blood vessels contain many leucocytes, mostly of the mononuclear type, embedded in the precipitated blue of the injection mass. This prussian blue precipitate in the blood vessels is not the coarse amorphous mass in which that color is usually seen under the microscope, but because the dye was thrown down in the presence of the colloidal serum proteids it is so finely divided as to appear homogeneous except when examined with the highest power immersion lenses with which its finely granular nature can be ascertained. It is in the same physical condition of finely divided suspension as the silver in silver gelatine mixtures of photographers, or the dye granules in injection masses made by precipitating colors in the presence of solidifying gelatin. The intracellular precipitates and those adherent to the surface of the omentum and its component fibres are much more coarsely granular.

The endothelial walls of both capillaries and larger vessels are stained a dark blue. The cytoplasm of the endothelial and mesothelial cells is entirely filled with a fine granular precipitate of the prussian blue, and in some places dye particles are found apparently between the cells, though the walls of the cells are in such close apposition that it is difficult to say with certainty that such is the cause. The cell nuclei are uncolored, and the cytoplasm of the serous mesothelium covering the omental surface is filled with the fine granules of dye which show the track of fluid which has passed through their bodies.

By the time that the dye bearing serum has reached the visceral blood vessels—liver, lung, etc.,—it has become so diluted with blood from non-absorbing parts of the body that it is not possible to follow the course of the chromogen through the body by the examination of sections. Large quantities are present in the kidney tubules, and the presence of the dye in the urine of the animal can easily be demonstrated.

The same conditions prevail, though they are much more difficult to demonstrate, in preparations made from omenta which have been immersed in strong solutions of trypan blue and collargol and colloidal solutions of other metals.

In animals which have been injected intraperitoneally with the chromogen solution—or where certain isolated portions of the peritoneal surface (small intestine or bladder)—have been immersed in, or covered with cyanide-citrate solution, and fixed in hydrochloric acid formalin, the blood vessels and lymphatics directly beneath the peritoneal surface, are found on section to be filled with dye precipitate and to have the same appearance as the omental vessels. Moreover it was possible in the gross to trace the stained lymphatic vessels directly to the lymph nodes into which they drained, and to obtain definite macroscopic evidence of the presence of prussian blue in the lymph gland. This feature of peritoneal absorption will be taken up separately in a later communication.

It is evident then from these histological preparations that there is very active absorption of foreign fluids through the peritoneal blood vessels, not only those in the omentum, but also through those beneath the peritoneum over the gut and bladder. In all probability fluids may be removed from the peritoneal cavity through any area in which blood or lymphatic vessels lie just beneath the peritoneal surface. Furthermore absorption of fluid obtains not only through capillaries, but through vessels of quite large caliber, and through arteries as well as veins, though not to as great an extent probably, because of the greater obstacles to fluid passage offered by the tissues which go to make up the thicker, denser arterial wall.

This is probably the reason for the pallor of the dye mass within the larger vessels, since it would seem reasonable to suppose that their thicker walls would hinder the passage of fluid and make it slower and of less amount. That fluids do pass through is shown by the fact that the wall and its lining endothelium contain granules of stain precipitated from the fluid during its passage into the vessels. That surrounding tissues have no significance in preventing fluid from coming in

contact with these large vessels is shown, as we have pointed out above, by the ease with which it penetrated between the cells in the perivascular fat and filled the capillaries by which the fat is supplied. There is of course the possibility of dilution of the chromogen fluid in the larger vessels as a result of their receiving blood from vessels through which absorption was not going on, but this is unlikely, since the vessels themselves and their entire tributary area were immersed in the test fluid.

The significance of the dark stained areas about the blood vessels is not quite clear. Apparently the solutions are drawn forcibly from the general tissue towards the blood vessels faster than they can be forced through the blood vessel wall, and then, removal being delayed (perhaps by the condensation of the connective tissue in the vascular margin) they are concentrated there. What the forces are which are exerted on the fluid, and what part is played by the movements of the omentum as a whole, the contraction of the blood vessels and the movement of the blood within them, it is impossible yet to say.

The material demonstrates also that while some fluid may pass between the lining cells of vessels on its way to their lumen, by far the larger part goes through the cytoplasm of the cells themselves. The sections are also of interest in that they show how little, if at all, the omentum was damaged during the operative procedure which preceded its immersion in the test fluid. Nowhere is there any sign of exudation or haemorrhage; there is no cellular death, as may be seen by the uncolored nuclei of the various cells; and, moreover, the mesothelial cells of the serous surfaces show no sign of disturbance or desquamation.

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A CASE OF A LEFT SUPERIOR VENA CAVA WITHOUT A CORRESPONDING VESSEL ON THE RIGHT SIDE

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TWO FIGURES

Many instances are recorded in the literature of the presence in human fetuses and adults of two venae cavae superiores, with or without a transverse inter-jugular anastomosis. The presence, however, of a left vena cava superior persisting without a right (the viscera not being transposed) is comparatively rare. I have studied the original descriptions of all the cases of this nature occurring in the bibliographies by Ancel, P. et Villemin ('08),¹ Boyd ('93), Halbertsma ('62), McCotter ('16), Nützel ('14), and Weigert ('81) with the exception of the case of Mäusert ('99) which was not available, and find that thirteen such cases have been previously recorded.

The subject of the anomaly here recorded is a middle aged, well developed male. The right internal jugular and subclavian veins unite to form a comparatively long innominate vein (referred to in this description as the right innominate) which extends obliquely downward and to the left, ventral to the roots of the arteries arising from the aortic arch and unites with the short left innominate vein to form the left vena cava superior. The left vena cava superior crosses ventral to the arch of the aorta, to the left pulmonary artery, and ventral to the root of the lung as it approaches the dorsal surface of the heart; here

¹ The case of Ancel, P. et Villemin ('08) is usually cited in the literature as being one of simple left vena cava superior, is one of a double vena cava. The case of Cheselden (1713) (the correct reference to which is given in the accompanying bibliography), has been difficult to find on account of the frequency with which an erroneous reference has been given.

it reaches the sulcus coronarius and becomes continuous with a large coronary sinus which opens into the right atrium in the usual situation.

A careful dissection was made for a vein representing the right vena cava superior, but no trace was found excepting the terminal part of the azygos which represents that part of the vena cava superior developed from the anterior cardinal. The highest right superior intercostal vein (draining the first space) is a tributary of the right vertebral. The azygos vein is somewhat smaller than normal, but receives the usual tributaries. It opens by means of the persisting caudal part of the anterior cardinal into the right innominate vein about one inch from its right extremity.

On the left side the highest intercostal is a tributary of the left vertebral vein. The uninterrupted hemiazygos system is a large vein (representing the left superior intercostal, hemiazygos and accessory hemiazygos) which opens into the left vena cava superior. Its caliber is nearly as large as that of the normal internal jugular vein. There are two inferior thyreoid veins (one for each lobe), each of which opens into the right innominate vein. The right internal mammary vein empties into the right innominate vein ventral to the termination of the azygos; the left is represented by two veins, the larger empties into the left vena cava superior, the smaller (representing the pericardiophrenic tributary) into the left innominate vein. The cardiac veins are normal in position and termination. The great cardiac is smaller than usual.

The heart is normal in size and position. In the upper dorsal part of the right atrium at the site of the ostium of the vena cava superior, the atrial wall is covered within by muscoli pectinati. Below this point the inner surface of the dorsal wall is smooth.

The ostium of the vena cava inferior at the lower and dorsal part of the atrium is normal, there is a faint trace of the inferior caval valve. The fossa ovalis and its limbus are normal, a foramen ovale being absent. Between the large coronary ostium and that of the vena cava inferior there is no intervening space, nor is there a coronary valve. Both from the exterior and in-

terior of the atrium, the two veins appear to communicate with the atrium by a common opening. The tricuspid valve is normal in position and arrangement. The remaining chambers of the heart are normal.

Before offering an explanation for this anomaly, the normal method of development of the veins in question may be briefly recalled. It is well known that the early embryological condition is one in which the veins are symmetrical on the two sides. On either side the anterior cardinal vein unites with the posterior cardinal to form the common cardinal vein (duct of Cuvier), and each common cardinal opens into the lateral part of the sinus venosus (sinus horn) of its own side. The sinus venosus, which is a transversely widened chamber, at first communicates with the common atrium by a large foramen, but during the formation of the interatrial septum, the sinus venosus comes to open into the right portion of the dividing atrium.

Somewhat later in development the terminal part of the subclavian veins (which at first open into the posterior cardinals) migrate cephalad to become tributary to the anterior cardinals. The large trunk on either side caudad of the confluence of the subclavian and anterior cardinal veins becomes the primitive vena cava superior. Each primitive superior cava consists of two regions, a cephalic part originally derived from the anterior cardinal and a caudal part derived from the common cardinal. The smaller trunks lying cephalad of the anterior cardinal-subclavian junction of either side represent the internal jugular of the adult.

Subsequently there is formed upon the medial side of each internal jugular vein, in close proximity to its junction with the subclavian, a vein (*Vena thymico-thyreoidea*, Thyng '14) which drains a venous plexus about the developing thyroid and thymic glands. An anastomosis between these thymico-thyroid veins evidently forms a transverse anastomosis, connecting the right and left jugulars, which normally becomes the *vena anonyma sinistra* (Szawlowsky '91, Anikew '09, and Thyng '14). This interpretation is substantiated by the fact that the inferior thyroid and thymic veins of the adult are usually tributary to the *vena anonyma sinistra*.

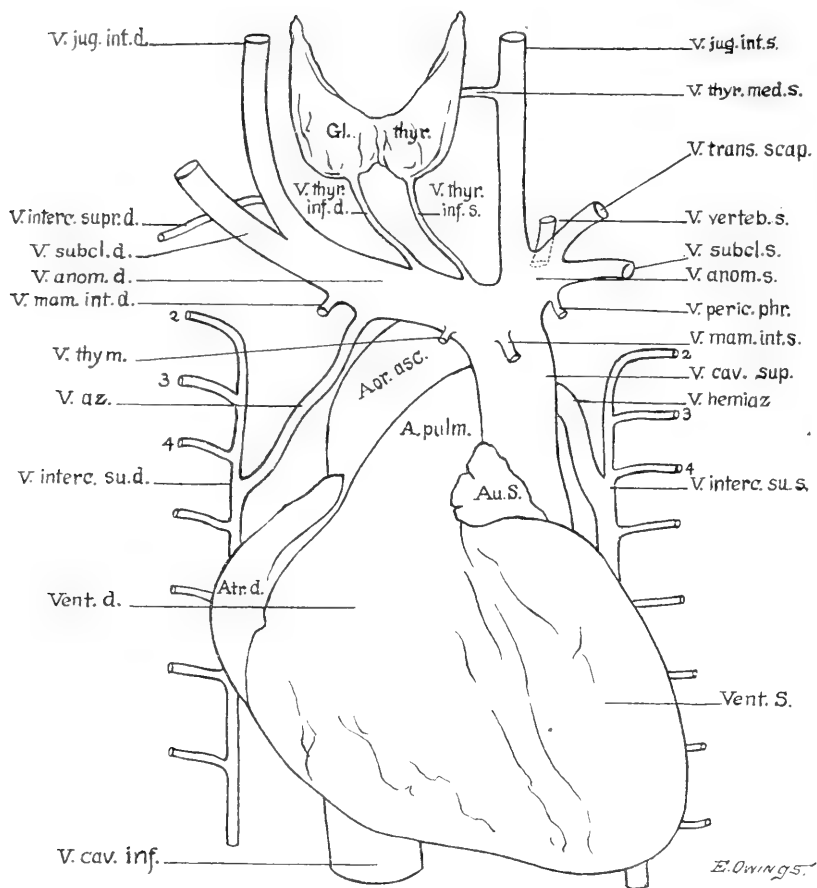


Fig. 1 Ventral aspect of heart and thoracic veins

ABBREVIATIONS USED IN FIGURES 1 AND 2

A. pulm., A. pulmonalis
Aor. asc., Aorta ascendens
Atr. d., Atrium dextrum
Gl. thy., Glandula thyroidea
V. anom. d., V. anonyma dextra
V. anom. s., V. anonyma sinistra
V. az., V. azygos
V. cav. inf., V. cava inferior
V. cav. sup., V. cava superior
V. hemiaz., V. hemiazygos

V. interc. supr. d., V. intercostalis suprema dextra
V. interc. su. d., V. intercostalis superior dextra
V. interc. su. s., V. intercostalis superior sinistra
V. jug. int. d., V. jugularis interna dextra
V. jug. int. s., V. jugularis interna sinistra

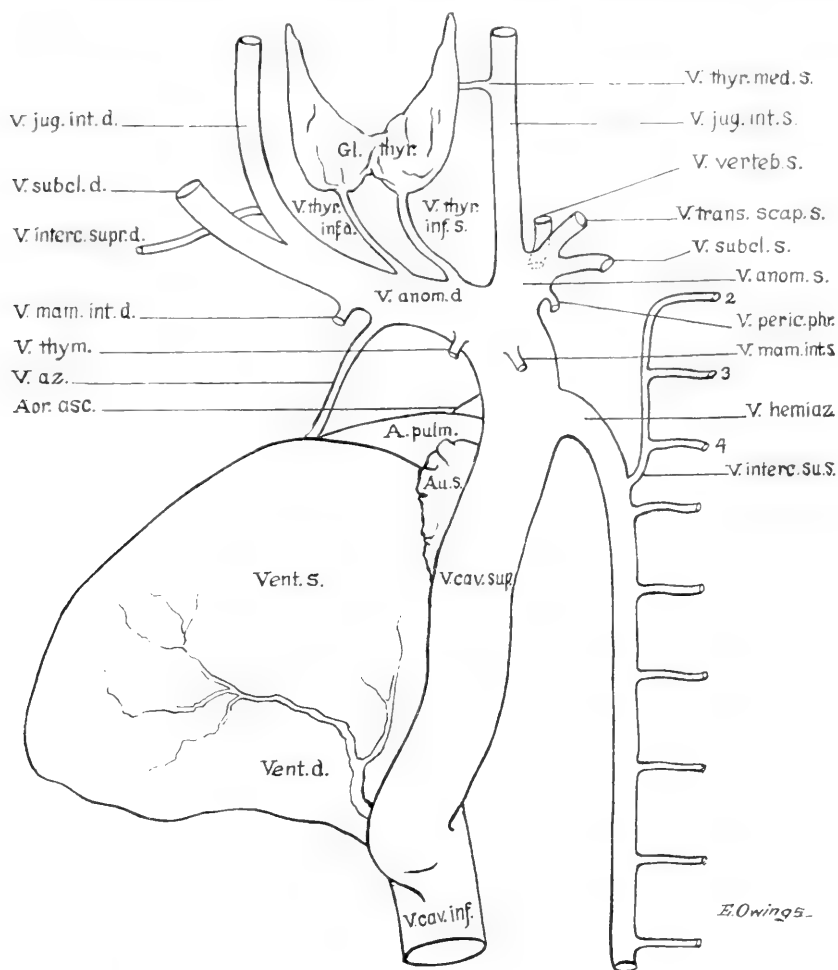


Fig. 2 Ventral aspect of thoracic veins. The apex of the heart has been turned toward the right to expose the left vena cava superior.

ABBREVIATIONS (CONTINUED)

<i>V. mam. int. d.</i> , V. mammaria interna dextra	<i>V. thy. inf. s.</i> , V. thyreoidea inferior sinistra
<i>V. mam. int. s.</i> , V. mammaria interna sinistra	<i>V. thy. med. s.</i> , V. thyreoidea media sinistra
<i>V. peric. phr.</i> , V. pericardio phrenica	<i>V. trans. scap. s.</i> , V. transversa scapulae sinistra
<i>V. subcl. d.</i> , V. subclavia dextra	<i>V. verteb. s.</i> , V. vertebralis sinistra
<i>V. subcl. s.</i> , V. subclavia sinistra	<i>Vent. d.</i> , Ventriculus dexter
<i>V. thym.</i> , V. thymica	<i>Vent. s.</i> , Ventriculus sinister
<i>V. thy. inf. d.</i> , V. thyreoidea inferior dextra	

Normally the blood which reaches the left side of the neck now presumably finds a more favorable course through the transverse inter-jugular anastomosis into the primitive right vena cava superior and thence into the right atrium, the greater part of the sinus venosus by this time having been absorbed into the latter. At any rate, the portion of the primitive left vena cava superior, representing the part of the common cardinal immediately caudal of the termination to the hemiazygos system (posterior cardinal), either atrophies or becomes fibrous. The transverse inter-jugular anastomosis then becomes the left innominate vein of the adult; the terminal part of the left anterior cardinal forms the proximal part of the left superior intercostal, and the caudal portion of the left common cardinal persists as the oblique vein which is tributary of the coronary sinus. The adult vena cava superior formed by the confluence of the right left innominate veins, represents the terminal part of the right anterior cardinal together with the entire right common cardinal vein.

EXPLANATION OF ANOMALY

The anomaly above described is apparently due to the fact that subsequent to the formation of the transverse inter-jugular anastomosis, the right common cardinal was obliterated instead of the cephalic portion of the left common cardinal, which here remained intact. This condition may be explained by assuming either that the left thymico-thyreoid vein had a more caudal origin than normally occurs, or that it migrated early in development to a more caudal position than is usual. In either case when the transverse inter-jugular anastomosis was formed, the blood, by following the course of least resistance, must have flowed from right to left instead of vice versa as usually occurs.

I wish to take this opportunity to extend my appreciation to Profs. H. D. Senior and F. W. Thyng of the University and Bellevue Hospital Medical College for their kind suggestions during the preparation of this paper.

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THE INNERVATION OF THE MUSCLE RETRACTOR OCULI

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ONE FIGURE

In view of the great number of dissections of the cranial nerves of the horse that presumably have been made in the veterinary colleges of this country and of Europe and in view of the probably still greater number of similar dissections of certain of our domestic animals, especially the dog, the cat and the rabbit that have been made in the numerous laboratories of comparative anatomy and physiology, it would seem that nothing further remained to be said concerning the gross anatomy of these nerves.

However, after many dissections of the cranial nerves of the horse and certain other of the domestic animals I am convinced that the descriptions of two of these nerves, viz., the N. oculomotorius and the N. abducens as given in many of the standard veterinary and comparative anatomies, are incorrect.

The error referred to consists in attributing two sources of nerve supply to the M. retractor oculi (retractor bulbi, suspensor oculi, posterior rectus, choanoid) namely, the N. oculomotorius and the N. abducens whereas the muscle is innervated exclusively by branches from the latter nerve.

The most common statement as to the distribution of the Nn. oculomotorius and abducens, in quadrupeds, is essentially that given by Chauveau as long ago as 1857. According to this author the N. oculomotorius is distributed to the following eye muscles—the dorsal, medial and ventral recti, the obliquus ventralis (or externus), the levator palpebrae dorsalis and the retractor oculi with the exception of its lateral portion; it also supplies one or more motor roots to the ciliary ganglion. The

N. abducens, according to Chauveau, supplies the M. rectus lateralis and the lateral portion of the retractor oculi. (Foltz states that Chauveau subsequently found that the M. retractor oculi was innervated exclusively by the N. abducens). This distribution of the two cranial nerves under discussion is, in the main, correct; the nerve supply to the M. retractor oculi however, as given by Chauveau and a number of other writers is without doubt incorrect.

A brief review of the innervation of the M. retractor oculi as given by several writers, in mammals and in some other animals will first be noted.

M'Fadyean gives precisely the same distribution as just quoted. Bradley's description is the same as the above with this slight difference, viz., the medial part only of the M. retractor oculi is mentioned as receiving a branch from the N. oculomotorius; the lateral portion of the muscle, according to Bradley, is supplied by a branch of the N. abducens, as described by Chauveau and M'Fadyean. In the latest American edition of Strangeway's Veterinary Anatomy no mention whatever is made of any portion of the M. retractor oculi being supplied by the N. oculomotorius. But taken in connection with what is said of the muscle "that it completely envelopes and forms a sheath round the extra cranial portion of the optic nerve" and also in connection with what is said concerning the distribution of the N. abducens "it is distributed to the lateral rectus and the lateral portion of the retractor oculi" one may fairly infer that the M. retractor oculi with the exception of its lateral portion, is supplied by some other nerve than the abducens, presumably by the N. oculomotorius.

In the first edition of his Veterinary Anatomy Sisson mentions both the oculomotorius and abducens as supplying branches to the M. retractor oculi; one portion of the muscle being supplied by a branch from the dorsal portion of the oculomotorius while the dorsal and lateral parts of the muscle are supplied by the N. abducens. In a subsequent edition, however, this error is corrected the M. retractor oculi being described as innervated by the N. abducens only.

According to Share-Jones the M. retractor oculi receives branches from three different sources—from the oculomotorius, the trochlearis and the abducens.

Martin and Franek state that the M. retractor oculi receives its nerve supply from both the dorsal and ventral branches of the oculo-motorius and from the abducens.

In contrast with this statement of Martin and Franek is that of Ellenberger and Baum, also Gurlt, who mention the dorsal branch only of the oculomotorius and the abducens as supplying branches to the M. retractor oculi.

Struska agrees in all respects with Ellenberger, Baum and Gurlt. Leisering says that the lateral portion of the retractor oculi is supplied by the abducens and the remaining portions of the muscle by branches from the oculomotorius.

According to Zimmerl the M. retractor oculi is supplied from the dorsal branch of the oculomotorius and from the abducens but he does not mention the particular portions of the muscle supplied from each of these two sources.

Varaldi gives practically the same distribution as Zimmerl only he does not state from which branch, dorsal or ventral, of the oculomotorius the filaments to the retractor oculi are given off.

In an article on the development of the eye muscles of the pig, Reuter describes the M. retractor oculi as receiving its nerve supply from both the oculomotorius and the abducens.

In the dog Bradley, Ellenberger and Baum describe both the dorsal and ventral portions of the N. oculomotorius as supplying branches to the M. retractor oculi; they make no mention of any branch from the N. abducens to this muscle.

In the second edition of *Anatomie des Kaninchen*, Krause describes and figures the ventral ramus of the N. oculomotorius as giving off a branch to the M. retractor oculi. Concerning the N. abducens he says that it gives branches to the M. rectus oculi posticus (rectus lateralis). In many mammals, he says the M. retractor oculi is supplied, as in the rabbit, by the N. oculomotorius; in the cat and the calf, however, the N. abducens is the source of the nerve fibers for the same.

Bensley gives precisely the same distribution of these two nerves in the rabbit as does Krause.

Reighard and Jennings say that in the cat the *M. retractor oculi* receives its nerve supply from the *N. oculomotorius* and that the *N. abducens* is distributed to the *M. rectus lateralis*; there is no intimation whatever that the latter nerve gives any branches to the *M. retractor oculi*.

Mivart, Wilder and Gage on the other hand describe the *M. retractor oculi* of the cat as supplied wholly by branches from the *N. abducens*.

Concerning the innervation of this muscle, Brinton says "the muscle which sweeps the broad nictitating membrane across the bird's eye and the funnel shaped or choanoid muscle (*retractor oculi*) which surrounds the optic nerve and eyeball of many mammalia are both supplied from this nerve (*N. abducens*).

Wiedersheim also describes the *N. abducens* as supplying the lateral rectus, the *retractor oculi* and the muscular apparatus of the *membrana nictitans* in sauropsida, thus agreeing in all respects with Brinton's account of the nerve.

From experimental evidence on the live horse and rabbit Foltz asserts that the *N. oculomotorius* supplies nothing to the *M. retractor oculi* but that the *N. abducens* alone supplies this muscle and the lateral rectus. In a note he further states "it is stated in the treatises of Veterinary Anatomy that this muscle (*retractor oculi*) in the domestic animals is animated chiefly by the common oculomotor." Chauveau has found recently and our experience confirms it, that this muscle is animated exclusively by the oculomotor externus (*N. abducens*).

According to Owen "in lower Quadrumana a few fibers seem to be detached from the inner part of the origin of the recti to be inserted into the sclerotic nearer the entry of the optic nerve. This is the remnant of a stronger muscle which in other mammals, with few exceptions, surrounds the optic nerve, expanding funnelwise, as it approaches the back of the eyeball; it is called the choanoid, muscle, or suspensor oculi, and is supplied by a branch of the sixth cerebral nerve."

Montane and Bourdelle describe and figure the N. oculomotorius as supplying all the muscles of the eye except the external rectus, the posterior rectus (retractor oculi) and the great oblique. The N. abducens they state supplies the external rectus and the posterior rectus.

Confirmatory evidence of the error of those who described the N. oculomotorius as supplying branches to one or more portions of the M. retractor oculi are found in the distribution of these two nerves in some of the reptiles. In a paper on the development of the musculature of the head and extremities of reptiles, Corning says that in the lizard (*Lacerta vivipera*) the abducens muscle mass which gives rise to the Mm. retractor oculi and rectus lateralis is supplied by the N. abducens. Precisely the same distribution of these two nerves is given by Hoffman for another lizard (*Lacerta agilis*) and for the turtle. In the Crocodile also, according to Fischer, the N. abducens is distributed as in the lizards.

In an investigation on the development of the prootic head somites and eye muscles in *Chelydra serpentina*, Johnson found that the N. abducens supplies the Mm. rectus lateralis and retractor oculi. No portion of the latter muscle is innervated by the N. oculomotorius.

The writer's conviction of the incorrectness of the descriptions of the distribution of these two nerves, as given by several authors, is based on repeated dissections of the nerves in the horse, ox, sheep, pig, dog, cat, and rabbit; in the woodchuck and the badger the nerves were dissected but once. In most cases the nerves were traced their entire length, i.e., from their superficial origin from the brain to their respective muscles. All of the dissections were made with the greatest possible care under a binocular dissection microscope. In the pig the nerves were also traced microscopically in a 42 mm. embryo cut into sections of 20 microns. In all of these animals the dorsal branch of the N. oculomotorius was distributed to the Mm. rectus dorsalis and the levator palpebrae; the ventral branch was distributed to the Mm. rectus ventralis, rectus medialis and to the obliquus ventralis. In all cases one or more small branches were given off

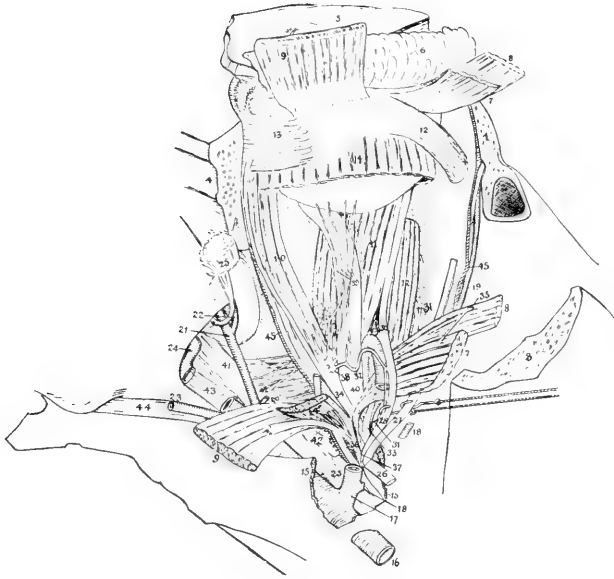


Fig. 1

1. Cut surface of supraorbital process.
2. Frontal sinus.
3. Cut surface of the zygomatic process of the temporal.
4. Cut surface of the malar.
5. Palpebrae.
6. Gl. lacrimalis (somewhat reflected).
7. M. rectus dorsalis or superior.
8. M. levator palpebrae dorsalis or superior.
9. M. rectus lateralis.
10. M. rectus ventralis or inferior.
11. M. rectus medialis.
12. M. obliquus dorsalis or superior.
13. M. obliquus ventralis or inferior.
14. M. retractor oculi or bulbi. (The greater part of the muscle has been removed; only the two extremities, 14 and 14' are shown).
15. Cut edge of sphenoid.
16. A. maxillaris interna.
17. A. ophthalmica.
18. A. temporalis profunda anterior.
19. A. supraorbitalis or frontalis.
20. Small artery to the mass of adipose tissue in the temporal ossa.
21. A. infraorbitalis.
22. A. orbitalis or malaris.
23. A. buccinatoria.
24. A. sphenopalatina.
25. N. maxillaris, cut and one end turned aside.
26. N. lacrimalis, cut and turned aside.
27. N. supraorbitalis or frontalis.
28. N. nasociliaris or palpebronasal.
29. N. ethmoidalis.
30. N. infratrochlearis.
31. N. trochlearis.
32. Sensory root of Ganglion ciliare.
- 33 and 34. N. oculomotorius, dorsal and ventral branches.
35. Small branch from the N. oculomotorius to the M. levator palpebrae dorsalis.
36. N. abducens.
37. N. orbitalis or zygomaticus (the peripheral portion has been removed).
38. Ganglion ciliaris.
39. Nn. ciliares.
40. N. opticus.
41. N. sphenopalatinus.
42. Ganglion sphenopalatinum showing many small nerves leaving it.
43. N. palatinus anterior or major.
44. N. palatinus posterior or minor.
45. Cut edge of the periorbita or ocular sheath.

to the ciliary ganglion. In no case were there found the slightest indications of filaments from either the dorsal or ventral branches of the N. oculomotorius to the M. retractor oculi as stated by so many.

In all cases the N. abducens, figure 1, 36, supplied all portions of the M. retractor oculi.

Most of the statements regarding the form of the M. retractor oculi and its relation to the optic nerve are somewhat misleading. In some of the domestic animals as the horse, ox, sheep and pig this muscle is not readily divisible into four distinct portions—dorsal, ventral, medial and lateral as it is in the dog, cat and rabbit, but forms a continuous sheet which surrounds the posterior part of the eyeball and a part of the extra cranial portion of the optic nerve. The medial side of the optic nerve for a distance of one and one half centimeters from the apex of the orbit, in the horse, is entirely uncovered by this muscle all the fibers of which are attached to the lateral side of the optic nerve as shown in the figure (14').

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THE ANATOMY WITH ESPECIAL CONSIDERATION OF THE EMBRYOLOGICAL SIGNIFICANCE OF THE STRUCTURES OF A FULL-TERM FETUS AMORPHUS

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NINETEEN FIGURES

INTRODUCTION

Although teratological literature is replete with descriptions of the various types of the fetus amorphus the specimen here studied is of sufficient interest to record, for no description, to which the author had access, was found that entirely coincides with this case. The outstanding characteristics of this interesting group of monsters are according to Ahlfeld ('04) the absence of a heart, normal bilateral symmetry and generally the brain. If the latter is present to any extent at all it is very rudimentary. The subcutaneous tissue is usually hypertrophied, cystic and oedematous. The cord possesses but one artery and one vein.

A partial list of the authorities consulted is the following: Ahlfeld, Ballantyne, Beardsley, Bevill, Brodie, Charlton, Claudius, Embleton, Hall, Herholdt, Houston, Hicks and Baukart, Hirst and Piersol, Jacobi, J. Jackson, J. S. B. Jackson, Le Cat, Lusk, Mall, Marchand, Meckel, Rauber, Schatz, Schwalbe, Simpson, Tiedmann, Von Winckel, Vrolik, Willey and Windle.

The specimen that is the subject of this paper was received by the author, upon taking charge of the department in 1914, from Dr. J. S. Foote, professor of histo-pathology, Creighton Medical College. Dr. Foote came into possession of the monster in 1901 shortly after it was born. It was well preserved in Kaiserling's fluid. The co-twin was a normal female fetus. The period of gestation lasted the full nine calendar months. The

normal fetus was the first to be expelled at parturition. No other clinical data were recorded; the condition of the fetal membranes and maternal deciduae were not tabulated. Ten years ago Dr. Foote heard that the normal co-twin was a healthy, robust girl of five years of age.

GENERAL APPEARANCES, WEIGHT AND DIMENSIONS

A systematic description of each aspect of the fetus follows in the text. The general appearance of the monster was that of an irregularly rounded, potato-shaped, skin-covered mass with the external indications of the two lower limb buds; a well marked outgrowth for the right upper limb and a slight protuberance over the region of the left upper limb. The ventral surface presented a thoracic and a hypogastric elevation. Upon the cephalic slope of the latter the umbilical cord was located which contained but one artery and one vein. The parietal, occipital and mid-dorsal regions carried hair. There was no external indication of a neck. No skeleton would be suspected by palpation. Upon percussion a tympanic note was elicited in the left lower thoracic region. There proved to be a large cavity, (11, fig. 18.) in this area, outside the skeleton, in the subcutaneous tissue. The facial and genital aspects, the limb buds and right glandular area were interesting from the embryological standpoint.

The specimen weighed 1130 grams after excellent preservation in Kaiserling's solution for fifteen years. The greatest length from the tip of the frontal region to the end of the rump was 19 cm. (4 to 16, fig. 2). The crown-rump measurement was 17 cm. (1 to 10, fig. 3). The greatest width through the upper limb buds was 13.5 cm. (7, fig. 4). Through the lower limb buds the width was 6.4 cm. The greatest thickness 8 cm. (5, fig. 3) was at the mid-ventral point on the line of greatest width. At the location of the external genitalia, the fetus amorphus was 4.5 cm. in thickness. On the ventral surface there were two elevations with an intervening furrow (6, fig. 3). The apex of the caudal elevation (8, fig. 3) was just below the umbilicus in the hypogastric region. The thickness of the embryo at this point was 6 cm.

SYSTEMATIC DESCRIPTION OF THE TOPOGRAPHY

Right ventro-lateral aspect (fig. 1)

The cephalic part of the fetus is inclined obliquely dorsad, so as to bring more prominently into view and to show the continuity and relationships of the lower limb buds, 12 and 13, and external genitalia; 9, body of the clitoris, 10, glans clitoridis, 11, ostium urogenital, 15, right labia majora, of the ventro-caudal aspect of the fetus, with the rest of the ventral surface

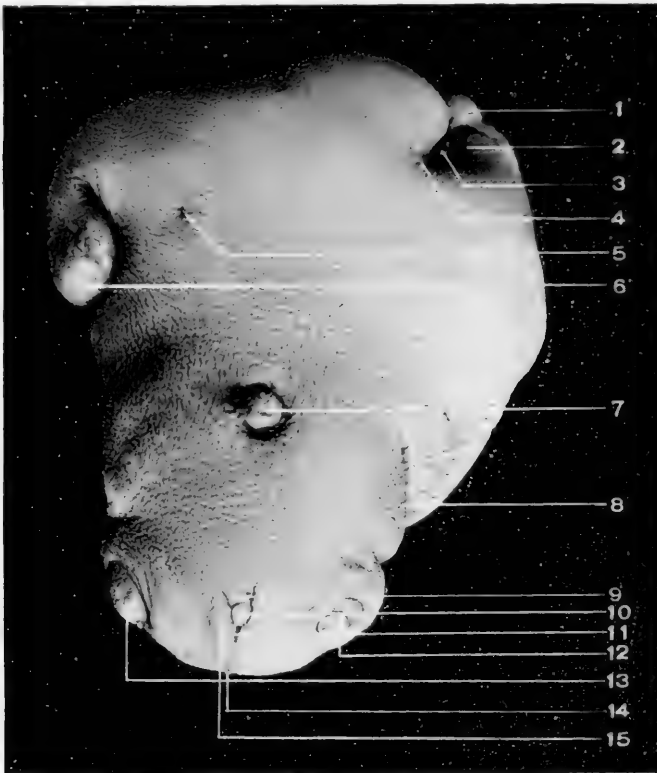


Fig. 1 (About one-half natural size.) 1, left globular process; 2, left maxillary process; 3, oral pit; 4, groove at ventral junction of mandibular arches; 5, depression of right glandular area; 6, right upper extremity; 7, umbilical cord; 8, the left ventro-latero-dorsal fissure; 9, body of the clitoris; 10, glans clitoridis; 11, ostium uro-genital; 12, left lower limb bud; 13, right lower limb bud; 14, imperforate anus; 15, the right labia majora; ventral aspect.

of the body. The embryological consideration of the structures of the external genitalia will be reserved until plate 2 is described. Directly cephalad from the genitals is seen the remnants of the umbilical cord 7, which contains only one umbilical artery instead of two as are normally found. In the right cephalo-lateral aspect is seen the marked protuberance of the right upper extremity 6. The slight protuberance, directly across the body on the left side, in the region of the left upper extremity is seen to a better advantage in figure 4, no. 7. The location of the imperforate anus is marked by 14.

The interesting fact to be considered in studying this aspect is the depression 5, of the right glandular area. No similar depression is seen on the left side of the body. This depression is a persistency of the depressed right glandular area, which appears in an embryo of about 25 cm., according to Pinkus ('10) and gradually deepens until it is well marked in a fetus of eight months. At this time the nipple is also supposed to be partly formed, but no sign of a nipple is seen in this specimen.

The genetic significance of the structures of the facial aspect; 1, left globular process, 2, left maxillary process, 3, oral pit, 4, groove at ventral junction of mandibular arches, will be discussed when plate 1 is considered, figures 6 to 10.

Left ventro-lateral aspect (fig. 2)

This view of the fetus is presented in order to show the continuity of the rudimentary facial structures with the ventral aspect of the thorax and abdomen. The cephalic part of the fetus is inclined obliquely ventrad, in contra-distinction to the position as shown in figure 1, in which the inclination was obliquely dorsad. The most important fact clearly presented is the absence externally of the neck separating head and thorax.

Dorsal aspect (fig. 4)

The left frontal region 1, is prominently marked. The hair is characteristically arranged over this area in converging whorls. On the other hand the hairs over the crown or vertex 4, are

arranged in diverging whorls, the direction of the hairs being toward the right, over the left side of crown as can be made out from the photograph. The literature on the subject of the whorls of the hair, together with a complete bibliography, is presented by Pinkus ('10). The external indication of the location of the superior sagittal suture is seen as a sulcus, 3. The

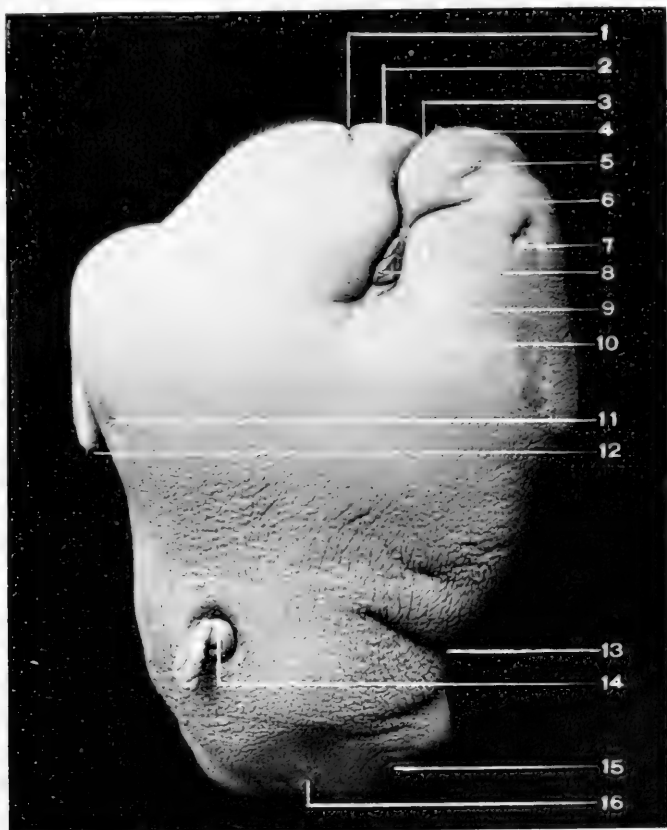


Fig. 2 (About one-half the natural size.) 1, right nasal pit; 2, right globular process; 3, sulcus between right and left globular processes; 4, ventral indication of prosencephalon; 5, left globular process; 6, left nasal pit; 7, palpebral folds of left eye; 8, tongue in oral pit; 9, left maxillary process; 10, ventral junction of mandibular processes; 11, depression for right glandular area; 12, right upper extremity; 13, left ventro-latero-dorsal fissure; 14, umbilicus; 15, left lower limb bud; 16, external genitalia, left ventro-lateral aspect.

right frontal and right crown regions are not so clearly differentiated morphologically in the fetus as the left regions. However, the converging and diverging whorls of the frontal and crown areas respectively, are seen, although they do not show so clearly on the right side as upon the left.

The normal and abnormal facial aspects (figs. 6, 7, 8, 9, and 10)

The abnormal facial structures of the monster are exceedingly interesting, due to the fact that they retain the external facial

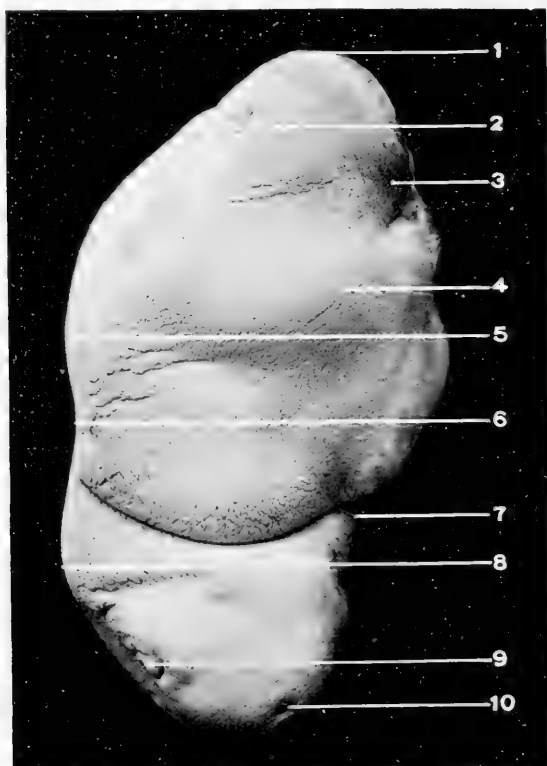


Fig. 3 (About one-half natural size.) 1, frontal area; 2, palpebral folds of left eye; 3, occipital area; 4, protuberance over left upper extremity; 5, cephalothoracic elevation; 6, ventral depression; 7, left ventro-latero-dorsal fissure; 8, ventro-caudal elevation; 9, left lower limb bud; 10, imperforate anus; left lateral aspect.

characteristics, however considerably distorted, of an embryo of the second month. In the light of normal facial development, as first satisfactorily studied by His ('80), and later by Keibel and Elze ('08), Hertwig ('06), Mall ('91 and '93), Retzius ('04) and others we are enabled to interpret the facts here presented.

Although considerably distorted, the two large globular processes 5 and 10, developed from the lateral aspects of the median

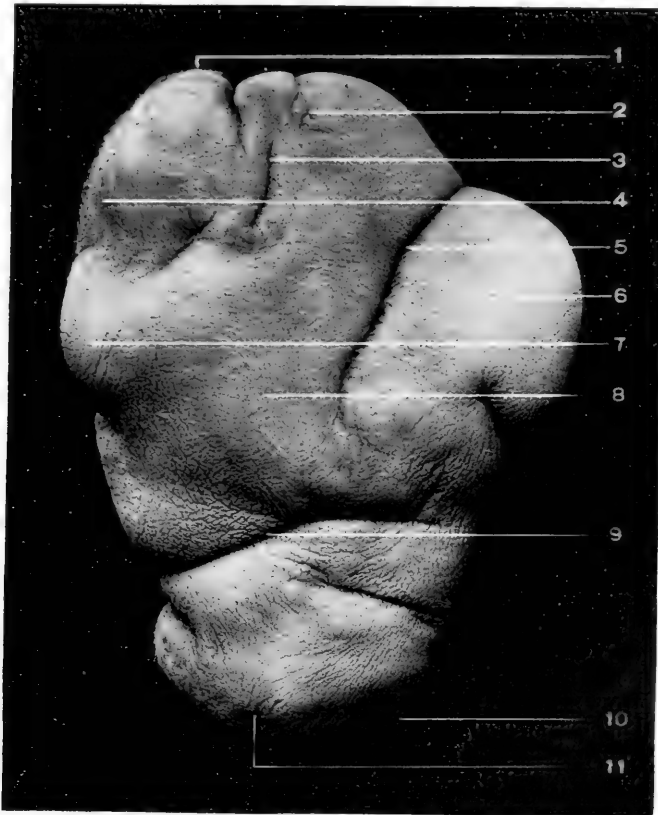


Fig. 4 (About one-half natural size.) 1, left frontal region; 2, palpebral folds of right eye; 3, superior sagittal sulcus; 4, occipital region; 5, deep furrow dorso-mesial of right shoulder; 6, right upper extremity; 7, protuberance of left upper extremity; 8, dorsal line of the body; 9, ventro-latero-dorsal fissure; 10, right lower limb bud; 11, imperforate anus; dorsal aspect.

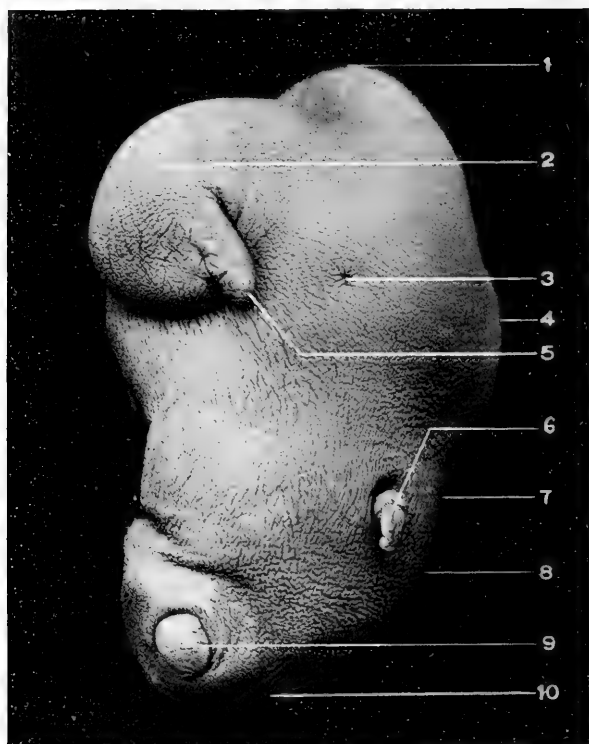
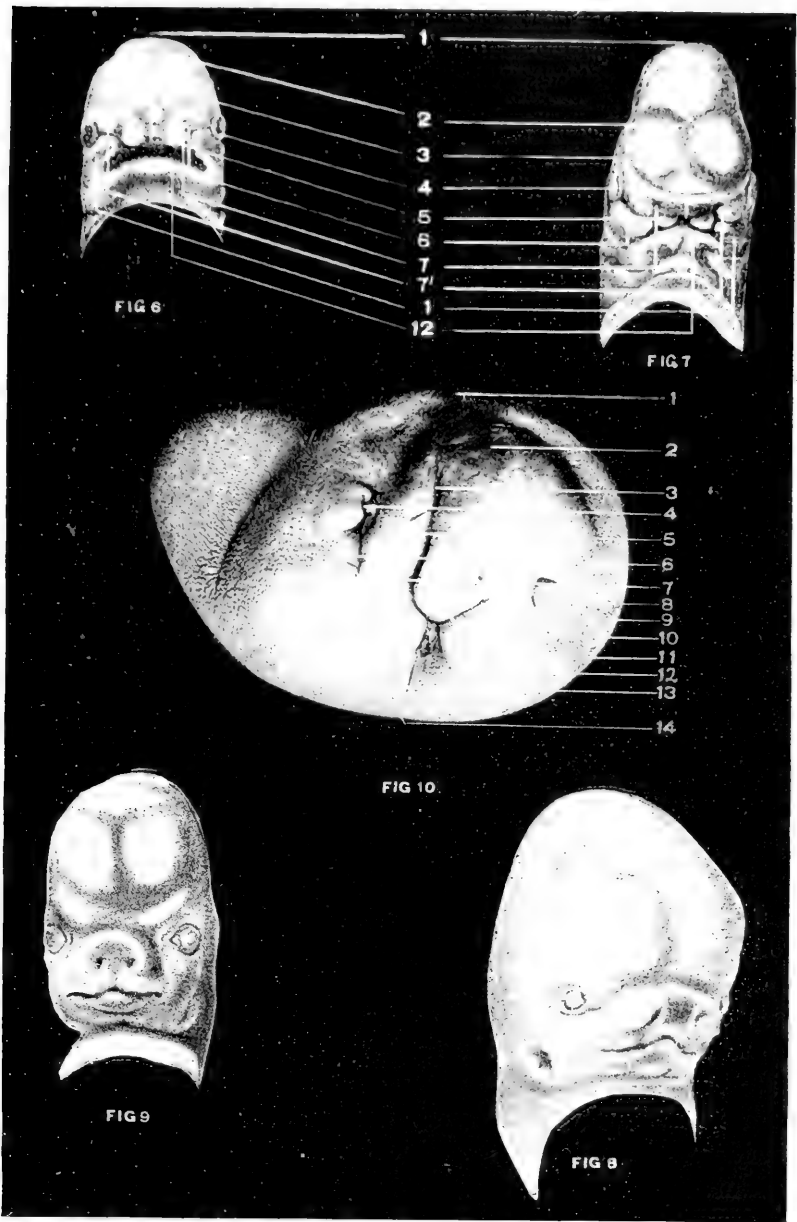


Fig. 5 (About one-half natural size.) 1, apex of right maxillary process; 2, right upper extremity; 3, depression of right glandular area; 4, ventro-cephalic elevation; 5, tip of the right upper extremity; 6, umbilical cord; 7, ventral depression; 8, ventro-caudal elevation; 9, button-shaped right lower limb bud; 10, location of external genitalia; right lateral aspect.

Figs. 6-10 The indication numbers of figures 6, 7 and 10 mark out similar structures. 1, crown; 2, frontal area; 3, sulcus between the right and left globular processes; 4, palpebral folds of right eye; 5, right globular processes; 6, nasolacrimal furrow; 7, nasal pit; 8, palpebral folds for left eye; 9, left nasal pit; 10, left globular processes; 11, left maxillary processes; 12, left mandibular processes; 13, ventral furrow intervening between mandibular arches; 14, right maxillary process; 7', in figures 6 and 7, lateral nasal processes. Figures 6, 7, 8 and 9. Development of the face of the human embryo by His taken from Heisler: figure 6, embryo of about twenty-nine days. The nasal frontal plate differentiating into processes globulares, towards which the maxillary processes of first visceral arch are extending. Figure 7 of about 34 days: the globular, lateral, frontal, and maxillary processes are in apposition; the primitive opening is now better defined. Figure 8 embryo of about the eighth week: immediate boundaries of mouth are more definite and the nasal orifices are partly formed, external ear appearing. Figure 9, embryo at the end of the second month.



nasal process are seen separated by a well marked fissure 3. The right and left nasal pits 7 and 9 respectively are shown as cicatricial depressions. The lateral nasal processes are not distinct on either side. However, their location is differentiated from the maxillary processes by the persistent naso-optic fissures best marked on the right side by 6. The palpebral folds of the right and left eye, 4 and 8 respectively, are clearly made out. The right is better developed and shows a fissure as does also the left, between upper and lower eyelids.

At the inner angle of the right orbit a groove or furrow continuous with the fissure between the two primitive eyelids is well marked, not so clearly seen on the left side, which courses to the lateral aspect of the anterior nares and is no doubt the persistent naso-optic fissure. The fissure is normally present as seen in figures 6 and 7, No. 6, but later becomes obliterated as seen in figure 9. On the left side the naso-optic fissure is not so well marked. The right and left maxillary processes, 14 and 11 respectively, are seen as prominent lateral bulgings of the cheeks. The external indication of the left mandibular arch 12, is apparent, separated by a narrow shallow furrow, 13, from the external indication of the right mandibular arch. This groove 13, leads cephalad to the distorted tongue, in the oral pit, shown darkly colored and protuberant in the photograph.

The primitive oral cavity is pentagonal in outline, bounded cephalad by the left enlarged globular process, caudad by the two indistinct external indications of the mandibular arches, and laterad by the external indications of the right and left maxillary processes. The facial aspect thus presents a double hare-lip due to lack of complete fusion of the maxillary and globular processes of their respective sides and the complete split-nose 3, due to lack of union of the right and left globular processes.

No bony nor cartilaginous structures were found in the mesoderm immediately underlying the superficial facial structures considered in this topographical description. No ears were present.

The normal and abnormal genital aspects (figs. 11, 12, 13, 14 and 15)

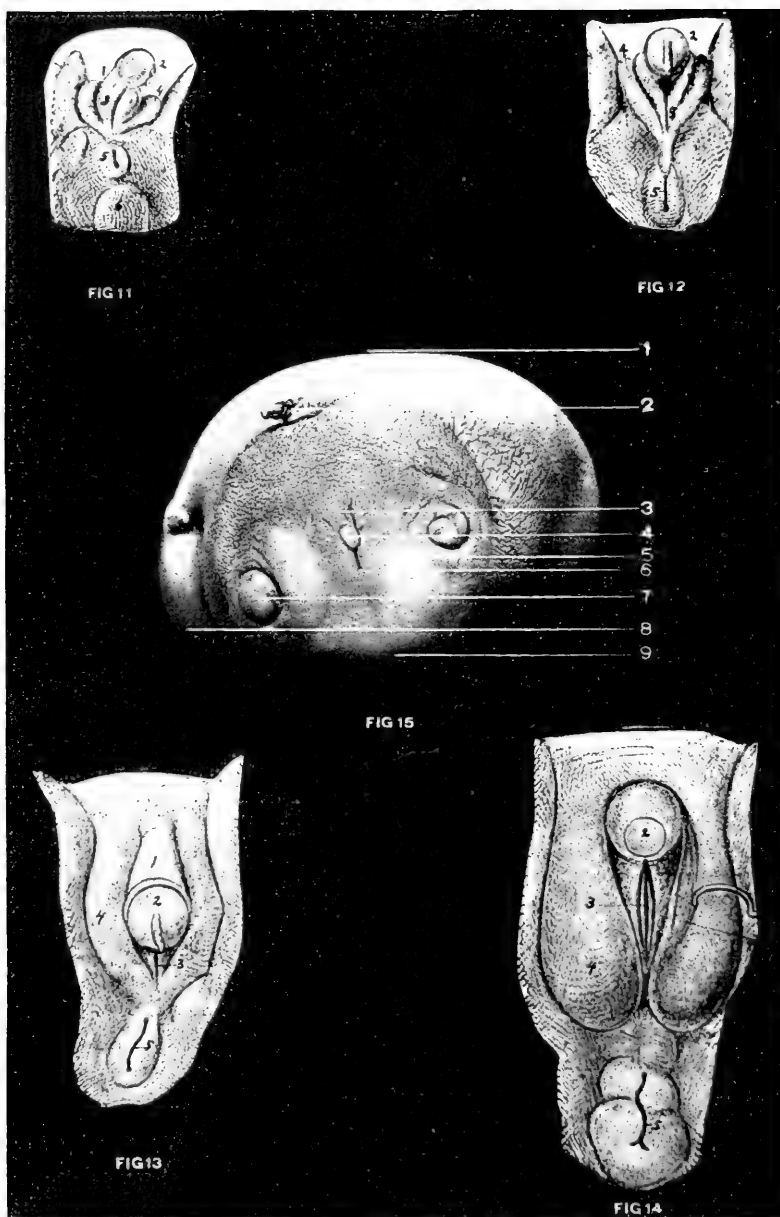
The structures of the genitalia of the monster are interesting, for to a certain degree they retain the characteristics of an embryo, however, considerably enlarged, at the beginning of the third month. At this time the indifferent phallus, by the direction which it takes, shows a transitory sexual differentiation. According to Herzog ('04), the phallus in the female bends downwards and in the male it is perpendicular to the long axis of the body.

By now referring to figure 15, we see an abnormally enlarged phallus 3, which has practically differentiated into the clitoris. The female phallus at the beginning of the third month may even be larger than that of the male; the downward direction as seen is diagnostic of the female embryo. Surmounting the phallus is the glans 4, at the base of which is an encircling fold, the praeputium. The urogenital sinus 6, is seen patent as a groove; the genital folds which bound this groove have receded inwards due to the overgrowth of the genital tubercles or swelling which now form the labia majora, 5. The urogenital opening distally, towards the glans clitoridis, on the anal slope of the phallus has closed and has formed the urethral groove. Proximally, towards the anus, the urogenital opening 6, remains patent, which is also diagnostic of the female embryo.

INTERNAL ANATOMY

Subcutaneous and muscular tissues

The fetus was opened by a median incision running to the left of the umbilicus. It was then observed that the skin was firmly adherent to the underlying dense oedematous connective tissue and fat. This tissue was less fibrous as the bony structures were approached. Frozen sections of the tissue contiguous to the skeleton were stained with hematoxylin and eosin. Isolated fibers of voluntary muscle were found intermingled with the connective tissue. There were no well defined groups of



Figs. 11-15 (Figs. 11, 12, 13, and 14. 1, phallus; 2, glans clitoridis; 3, ostium urogenital; 4, labia majora; 5, anus; 6, coceygeal eminence; 7, labia minora. Fig. 15 1, ventral surface of fetus amorphus; 2, umbilical cord; 3, body of the phallus; 4, glans of clitoridis; 5, left labia majora; 6, urogenital sinus; 7, right lower limb bud; 8, dorsal aspect of right upper limb; 9, imperforate anus.

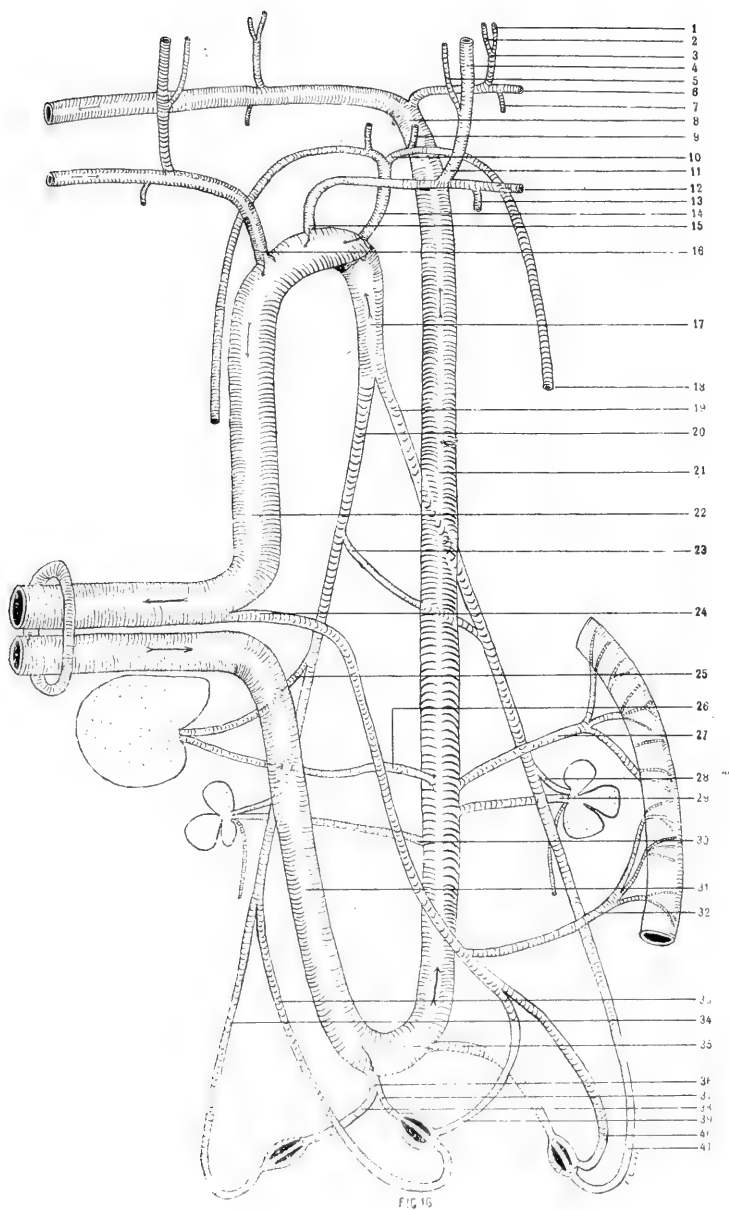
musculature. In the irregularly arranged muscular fibers were seen various sized vacuoles, which when stained in an alkaline alcoholic solution of scarlet red fat stain, which is Bell's modification of the Herheimer method, proved to be fat. Counter staining was made with Delafield's hematoxylin and the sections were mounted in glycerin. This method proved an excellent one for the detection of fat within the muscular fibers. The fat vacuoles were found in the angles of Cohnheim's areas and in the spaces between the individual sarcostyles.

Blood vascular system (fig. 16)

The chief features of the blood vascular system of the fetus amorphus are the absence of a heart and, according to Ahlfeld ('80 and '82), the reversal of the flow of blood. In the diagram the arrows indicate the reversed flow of blood according to Ahlfeld's theory. However this idea is refuted by Breus ('82).

The blood enters the body of the fetus amorphus through its single umbilical artery 31, figure 16. As the latter turns to become continuous with the dorsal aorta, there are two branches given off, 35 and 36, the left and right common iliacs, respectively. The single umbilical artery is seen to belong to the right side. There is no left umbilical artery present. The right common iliac 36, branches into the internal and external iliac arteries, 37 and 38 respectively. The internal iliac artery ends as shown diagrammatically in figure 16 in a dilatation representing the capillaries of the pelvic viscera. This vessel evidently carries the blood supply to the viscera as no left internal iliac vessel is present. The right external iliac is also shown diagrammatically as ending in a dilatation representing the capillary system of the right caudal limb bud. The left common iliac 35, pursues a straight course to the left caudal limb bud where it breaks up into a capillary network.

The next vessel given off as we follow the blood current up through the aorta is the right renal artery 30, next the left renal 29, and lastly the hepatic artery 26, which comes off on the ventral surface of the aorta. At the same level, on the



left lateral aspect, to the origin of the hepatic artery, the superior mesenteric artery 27, is found. There was no vessel comparable to the inferior mesenteric. The coeliac axis is represented by the single trunk of the hepatic artery. The superior mesenteric artery is a good sized trunk and evidently supplied the regions of the intestine that are normally supplied by the coeliac axis and the inferior mesenteric arteries as well as its own region.

At 8, the aorta is seen to bifurcate. The right trunk is very much the larger of the two. The branches given off from these two trunks are comparable on each side. The first branch on the left side is the common carotid artery 3, which bifurcates into the external 1, and internal 2, carotid arteries. Caudad the internal mammary artery is given off, 7, from the subclavian, 6. From the dorsal aorta the paired intercostal and lumbar arteries which are not represented in the diagrammatic reconstruction, are given off.

The blood is returned from the left and right sides of the head and neck and upper extremities through the left and right superior vena cava 15 and 16, respectively. The left external 4 and internal 5, jugulars unite to form a common trunk which in turn with the left subclavian vein 12, unite to form the left superior vena cava 15. At the junction of the two veins men-

Fig. 16 1, external carotid artery (left); 2, internal carotid artery (left); 3, common carotid artery (left); 4, left external jugular vein; 5, left internal jugular vein; 6, left subclavian artery; 7, left internal mammary artery; 8, dorsal aorta; 9, vertebral vein; 10, point of fusion of left and right azygos veins; 11, trunk of left common jugular vein; 12, left subclavian vein; 13, left internal mammary vein; 14, common trunk of azygos veins; 15, left superior vena cava; 16, right superior vena cava; 17, common trunk of inferior vena cava; 18, left azygos vein; 19, left inferior vena cava; 20, right inferior vena cava; 21, thoracic aorta; 22, umbilical vein; 23, an anastomotic vein between left and right inferior vena cavae; 24, diagonal branch of umbilical vein draining the intestinal pelvic, and left lower limb buds; 25, the hepatic vein; 26, the hepatic artery; 27, superior mesenteric artery; 28, left renal vein; 29, left renal artery; 30, right renal artery; 31, umbilical artery; 32, superior mesenteric vein; 33, vein draining pelvic region into right inferior vena cava; 34, continuation of femoral vein with the right inferior vena cava; 35, left common iliac artery; 36, right common iliac artery; 37, internal iliac artery; 38, external iliac artery; 39, vein draining pelvic area into diagonal branch of umbilical vein; 40, vein draining left lower limb bud into diagonal branch of umbilical vein; 41, left inferior vena cava.

tioned above to form the left superior vena cava, the left internal mammary vein 13, joins the left subclavian. The branches forming the trunk of the right superior vena cava are similar to those forming the left superior vena cava but they are not labeled in the diagram.

The left vertebral vein is represented by 9; the right is not labeled. The regions drained by the left superior intercostal vein and the azygos system of veins are represented by 18. The right side is not labeled but is similar to the left. The vessels of the two sides after receiving the left and right vertebral veins form a common trunk 14, which flows into the arch of the umbilical vein. There are no pulmonary veins as well as no pulmonary arteries. The blood is returned from the pelvic viscera through a vessel 33, which empties into the right inferior vena cava and a vessel 39, which empties into a diagonal branch 24, which in turn pours its blood into the umbilical vein shortly before it enters the umbilical ring.

The blood is returned from the left caudal limb bud by two veins, one 40, which goes to form with the left pelvic visceral vein 39, the diagonal vein 24, and one 41, which is continuous with the left inferior vena cava. From the right caudal limb bud there is but one vein 34, which joins the vein 33, draining the pelvic viscera. These two latter veins unite to form the right inferior vena cava and no doubt represent the right and left common iliac veins. Upon the left side the left inferior vena cava does not bifurcate into right and left common iliac veins as is found on the right side. The right inferior vena cava receives the right renal and hepatic veins, 25. The left inferior vena cava receives the left renal vein 28. The two inferior venae cavae 19 and 20 unite to form a single trunk which empties into the dorsal aspect of the arch of the umbilical vein. Before these two veins unite there is an anastomotic branch 23, coursing cephalad from left to right. The blood draining the intestines empties through the superior mesenteric vein 32, into the diagonal vein 24, which in turn pours its blood into the common vein of exit, the umbilical vein, 22.

The abnormal reversal of the direction of the blood stream, through a single umbilical artery, with its subsequent accentuation of certain arteries and degeneration of others, increases the difficulty of interpreting the significance of the vessels dissected. The arch of the aorta is obliterated and at its location we find a bifurcation, with the right integer which supplies mainly the right upper limb, considerably the larger of the two. The left 15, and right 16, superior venae cavae are the persistent left and right anterior cardinal veins. Instead of the two posterior cardinal veins joining their respective anterior cardinal veins, they join each other to form a common trunk 14, which empty into the umbilical venous arch, and is no doubt a persistency of the sinus venosus of the embryonic heart. The inferior vena cava is double 19 and 20, up to within one inch of its termination, in the umbilical venous arch where its two components of the right and left sides fuse to form a common trunk, 17. These two posterior venae cavae are undoubtedly the persistent remains of the two subcardinal veins.

There is no portal system comparable to that found in the normal adult. The small rudimentary liver possesses but one artery and one vein. The diagonal vein 24, draining the blood from the intestines, pelvic viscera and left limb bud is evidently the remains of the portal system. The blood through this vein empties directly into the umbilical vein.

Respiratory system (fig. 17)

The lung tissue was completely degenerated. In the thorax there was found a putty-like mass of degenerated tissue through which the oedematous connective tissue was growing. Broken off portions of the cartilaginous bronchi were found throughout the above mass. The small bronchi which contain portions of cartilage and those in the stage of pre-cartilage, proved to be very resistant. The trachea 3, is seen tapering to its bifurcation into the right and left bronchi at 5. There were rudimentary respiratory passages with anterior and posterior nares leading into the dorsal aspect of the deformed mouth. The larynx 1, is considerably dilated as seen in figure 17.

Digestive system (fig. 17)

The alimentary tract began at the mouth, and at the caudal part of the pharynx dorsad to the larynx the esophagus 2, figure 17, began. A small dilatation 6, is detected in the region of the stomach. A duodeno-pyloric flexure is made and at the base of the umbilical ring 7, and extending into the umbilical cord, a few coils of small intestine 9, were unraveled. The small cecal evagination 8, was found at the base of the umbilical ring. The large intestine had already made the primary twist across the small intestine. From the cecum the large intestine 13, is traced to its termination in the rectum 25. The descending and part of the transverse colons are marked out at this stage of development. The relationships of the intestinal coils to the umbilical vein 10 and umbilical artery 11, and urachus 12, at the base of the umbilical ring, are also shown in figure 17. There was no pancreas present. The common bile duct was absent. The rectal cul-de-sac is connected to the utero-vaginal tube by a fissure-like opening 22.

Uro-genital system (fig. 17)

The urinary apparatus is separated from the genital structures as shown in the figure. Both kidneys 16, are tri-lobed, the left being slightly larger than the right one. A ureter 17, leads from each to the bladder, 21. From the bladder leading to the uro-genital sinus is the urethra 23. From the tip of the bladder there is a patent tube, the urachus 12, which leads up to and out through the umbilical cord.

Fig. 17 1, The larynx; 2, the oesophagus; 3, trachea; 4, broken off bronchi in thorax; 5, bifurcation of trachea into the right and left bronchus; 6, spindle shaped stomach; 7, umbilical ring; 8, cecum; 9, loops of small intestine; 10, umbilical vein; 11, umbilical artery; 12, urachus; 13, descending colon; 14, left renal vein; 15, left renal artery; 16, tri-lobed metanephros; 17, left ureter; 18, fimbriated end of left Fallopian tube; 19, left Fallopian tube; 20, dichotomus division of the uterus into the two Fallopian tubes; 21, bladder; 22, opening between rectal cul-de-sac and the utero-vaginal tube; 23, urethra; 24, vaginal portion of ureto-vaginal tube; 25, distal end of rectal cul-de-sac.

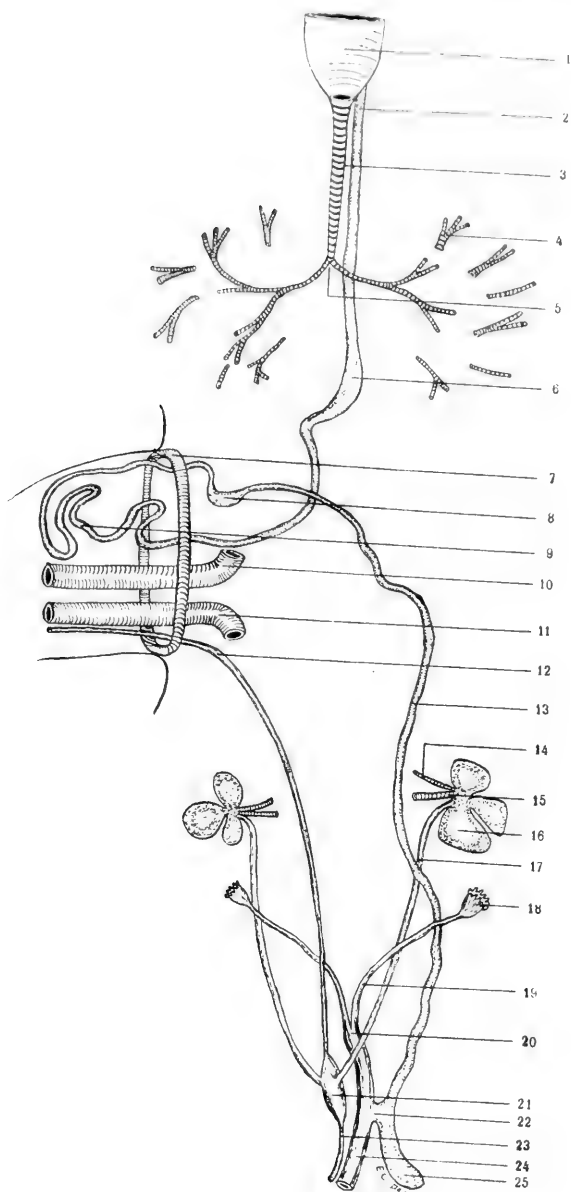


FIG 17

The genital system is composed of a utero-vaginal tube 20 to 24, continuous with the two oviducts which branch immediately in a Y-shaped manner. The latter end in a fimbriated dilation 18, the left slightly larger than the right. There were no ovaries. The alimentary tract is connected with the utero-vaginal tube at the location marked 22, which represents the persistent connection between the alimentary tract and the urogenital systems in the cloaca.

Nervous system

There was an entire absence of the brain and cranial nerves. The upper part of the cord was absent as far as the seventh cervical nerve. From this point to the end of the lumbar vertebra the cord was present. There was a rudimentary cauda aquina extending from the caudal end of the cord. There were irregular net works of nerves in the regions comparable to the brachial and lumbo-sacral plexuses.

Skeletal system

From the tip of the frontal bone, the highest point on the skull, to the tip of the fourth sacral vertebra the bony skeleton measured 14.5 cm. (2-12, fig. 19) From the tip of the left shoulder girdle to the tip of the rudimentary right ulna along indication line 9, figure 18, the width was 8.5 cm. A comparison of the intact fetus amorplus can easily be made with its skeleton when it is remembered that before the dissection the monster measured 19 cm. greatest length and 13.5 cm. the greatest width. At least 2.5 to 3 cm. of dense connective tissue and fat were interposed between the tip of any bony structures and the outer skin which precluded the palpation of the underlying skeleton.

The skeleton was composed of a deformed cranium which was composed of, a fused occipital, sphenoid, ethmoid, temporals, and frontals. The two parietals were respectively distinct. Many of the bones of the face and those of the bony vault of the cranium were fused into a distorted mass and could be made

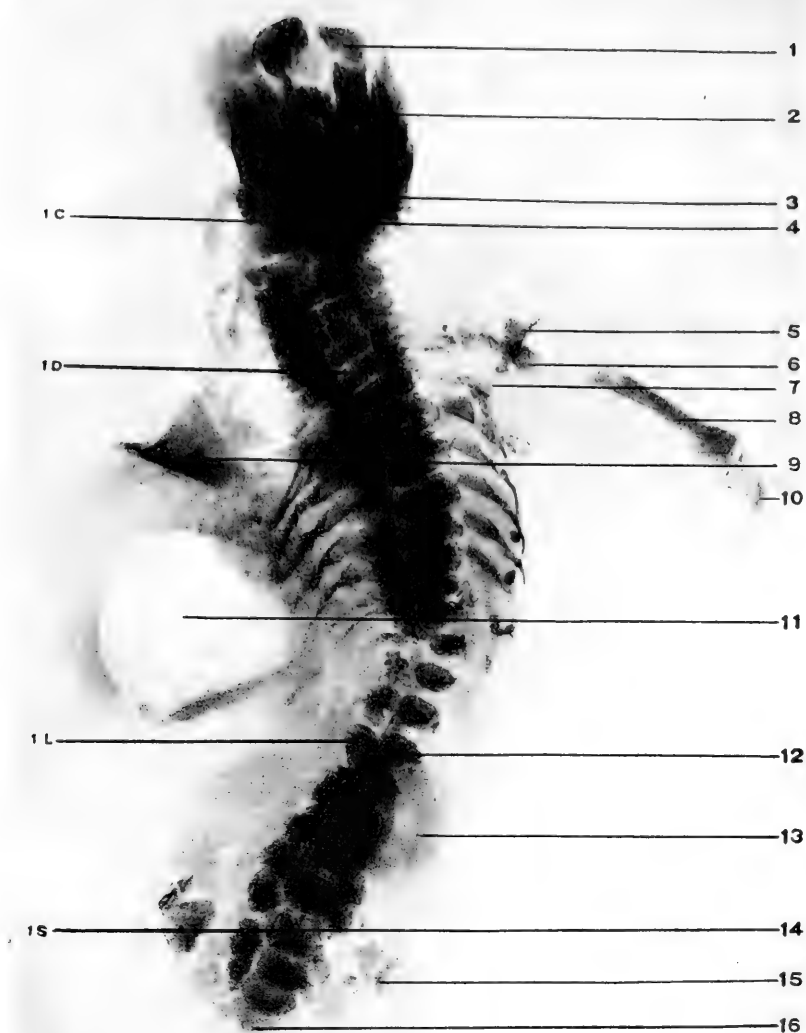


FIG 18

Fig. 18 Skiagraph No. 1. 1, right parietal bone; 2, right temporal bone; 3, occipital bone; 4, atlas bone; 5, spine of scapula; 6, glenoid fossa; 7, right clavicle; 8, right humerus; 9, left scapula; 10, proximal end of ulna; 11, left subcutaneous cavity; 12, first lumbar vertebra; 13, liver tissue; 14, first sacral vertebra; 15, center of ossification of right ilium; 16, fourth sacral vertebra; 1c, first cervical vertebra; 1d, first dorsal vertebra; 1L, first lumbar vertebra; 1s, first sacral vertebra.



FIG 19

Fig. 19 Skiagraph No. 2 1, fused mandible and temporal bones; 2, frontal bone; 3, right parietal bone; 4, anterior fontanelle; 5, left parietal bone; 6, occipital bone; 7, styloid process of left temporal bone; 8, left clavicle; 9, liver tissue; 10, first lumbar vertebra; 11, first sacral vertebra; 12, center of ossification of left ilium.

out only with difficulty and then only by location and vague similarities to the normal bones. The nasal, lacrimal and vomers were completely fused and these in turn were fused to the mid-point of the mandible by the plowshare of the vomer. The mandible was completely continuous by bony union with the temporal bones on each side of the cranium. The paired maxillae, zygomatic and palate bones, six in all, were entirely absent. A rudimentary hyoid bone was present.

The vertebral column presented complete spina bifida from the atlas to the fourth sacral vertebra 16, figure 18. The laminae were widely separated which deformity is well seen in figure 18 in the dorsal view of the skeleton. In the cervical, most of the dorsal, lumbar and sacral regions the laminae were fused to each other. The atlas was completely fused with the occipital bone at the base of the skull. As can readily be seen by reference to figure 18, the vertebrae presented right lateral scoliosis with the vertex of the curvature at the ninth dorsal vertebra. There is also a slight dorsal kyphosis, sharply angular, from the second to the sixth dorsal vertebrae, somewhat obscured by the opacity in figure 19. The last sacral and coccygeal vertebrae were absent.

Upon the left side of the thorax the first nine ribs were fairly well formed with the remaining three merely attenuated cartilaginous rods. Upon the right side the first seven ribs were fairly well formed and the remaining five represented only by fine rods of cartilage. Upon the ventral aspect the thorax presented a complete cleft sternum. The cartilaginous ribs over the cardiac area presented a distinct bulging.

Upon the left side the upper extremity presented a compound clavicle and scapula the remaining bony structures being absent. Upon the right side in addition to the distinct clavicle and scapula (which are not compound as on the left side) there was a well formed humerus 3 cm. long and the proximal end of the ulna 0.5 cm. long. No radius nor other bones of the upper extremity were present. Both clavicles were completely ossified. The left scapula presented ossification along the acromial process, where it is continuous with the left clavicle. The spine

and supra-spinous fossa were also completely ossified. The corocoid process was not present. The right scapula was completely cartilaginous except the acromial process and contiguous parts of the spine and corocoid process.

The pelvic girdle presented centers of ossification for the ilium, ischium and pubis. The sacro-iliac synchondrosis was cartilaginous on its iliac and sacral aspects. Both pubic elements of the symphysis were cartilaginous. Besides the pelvic girdle there were no other bones of the lower extremity present.

In conclusion, I wish to express my gratitude to Dr. J. S. Foote for the fetus amorphus recorded in this article; to Prof. J. C. Heisler and the Saunders Publishing Company for their permission to reproduce figures 6, 7, 8, 9, 11, 12, 13 and 14 of this article from Heisler's *Embryology*; and to Dr. A. F. Tyler, professor of Roentgenography, Creighton Medical College, for the skiagraphs herein reproduced.

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THE THORACIC DUCT IN THE RABBIT

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SIX FIGURES

Notwithstanding the above title I intend to describe not only the thoracic duct, its beginning and termination, but also the large lymphatic vessels in the abdominal cavity which are its real beginning. In my description I will also take into account the main lymph channels from the brim of the pelvis.

The injections were made on a series of rabbits, the exact species of which because of cross breeding I was unable to determine. They were bought at random from rabbit farms where they had been bred promiscuously. I used 26 adult animals about two years of age, sixteen males and ten females. Immediately after killing by illuminating gas the animals were taken from the jar and placed on an animal board. The hind and front foot pads were immediately injected by means of an ordinary hypodermic syringe with a medium-sized needle. I used India ink for the injection mass and found that by inserting the needle just under the skin in the pad of the foot, this fluid would immediately travel on up the leg in the lymphatic vessels. Sometimes the ink would go only as far as the nodes in the popliteal fossa, while in other animals it would go clear up into the abdominal vessels, and even into the thoracic duct. Out of the 26 animals I succeeded in getting the injection fluid to go from the pad of the hind foot clear up the thoracic duct in five cases. In all the rest it stopped at the popliteal nodes. In three of the five cases I found that there was an anastomosis of the vessels around the popliteal node but in the other two cases it looked as though the injection had gone right through the node. The inner part of the node was usually darker than

the periphery and in one instance just one portion of the node was blackened. Whether this can be explained by a difference in the arrangement of the lymphatic vessels or is due to the effect of the strong pressure created by the syringe I cannot say. However, my classmate, Clattenberg, found that young kittens and guinea pigs, in same way, always show the injection clear up the thoracic duct.

It occurred to me that perhaps I did not have enough pressure to force the injection on through. I therefore used a larger syringe, in a few cases, and injected as much as I could into the subcutaneous tissue, but the results were as before for I did not get the injection to go beyond the popliteal nodes in any of these cases.

After injecting the pad of all four feet in this manner I tied the animal to the board and made an incision through the skin from the mandible to the xiphoid. I then dissected out the two external jugular veins, which are very large in the rabbit and ligatured them close to the clavicle, and opened the thorax and tied off the two superior venae cavae. In this way I prevented the injection mass from either going on into the heart or cranially through the large veins. Next I skinned the hind leg and dissected out the popliteal nodes. There may be one or two of these and they are of considerable size. Upon injecting these nodes the ink usually went through into the thoracic duct very easily if the leg was repeatedly flexed and extended.

I also always opened the abdomen and injected the large group of mesenteric nodes in order to distend the duct and vessels so that they stood out distinctly. Injections into the pads of the anterior extremity usually stopped in the axillary nodes. I succeeded in injecting these nodes only a few times and not as easily as the popliteal nodes. In almost every case about 14 or 15 cc. of ink was used per rabbit. Of this 4 cc. was injected from the hind and 3 cc. from the front pads, 4 cc. from the popliteal nodes, 1 to 2 cc. from the axillary nodes and 3 cc. from the mesenteric nodes. Since I was not able to dissect the vessels very well in the fresh specimen the whole animals were preserved in a dilute solution of formalin. After this I could

finish my dissection easily especially that of the duct at its termination into the great veins of the neck.

The injection went through the nodes at the bifurcation of the abdominal aorta to the vessels above. This raises the question as to whether an injection can go through a node. This Baum '11 answered in the affirmative. Baum also always found connections of the lymphatics with the peripheral veins but this was never noticed in my injections.

In my series of animals I found that the thoracic duct and abdominal vessels were very constant and similar in distribution and appearance for only minor variations were observed. Hence a single scheme can represent the arrangement in all the specimens injected with the exception of a few variations which will be spoken of later.

From 3 to 5 lymph nodes were always found at the brim of the pelvis or better at the bifurcation of the large abdominal blood vessels. The more usual number was three nodes. One lay on each common iliac and the third ventral to the bifurcation of the abdominal aorta. However, the position of these nodes varied somewhat for the third or central one was sometimes found in the pelvis just caudal to the bifurcation of the aorta and the iliac nodes also sometimes lay more caudal. The nodes lay in the subperitoneal tissue always ventral to the vessels and could be moved about very easily. They varied in size from 0.5 cm. to 1.2 cm. in diameter and were connected together by vessels thus forming a kind of a plexus.

From this group of nodes a group of 3 to 7 lymph vessels started cranially but in every case small branches of these vessels would connect with the adjoining vessels or a more dorsal vessel so as to form a sort of plexus. Nevertheless I could almost always distinguish and trace a certain number of distinct and parallel vessels which ran cranially as far as the renal vein. This group of lymph vessels was always mainly ventral to but partly surrounded the great abdominal blood vessels. The number and exact position of these lumbar lymph vessels and their relation to the vein and artery I have tabulated in the accompanying table. The average number of lumbar

TABLE 1

NUMBER OF ANIMAL	NUMBER OF NODES AT BIRTH OF PELVIS	NUMBER OF LYMPH VESSELS FORMING THE DUCT					NUMBER OF THORACIC DUCTS AT TERMINATION	NODE PRESENT TO RIGHT IN UPPER THORAX	VARIATION AT OR NEAR TERMINATION	PLACE OF TERMINATION		
		In abdominal plexus	Ventral to vein	Ventral to artery	Dorsal to artery	Cranial cisterna				External Jugular	Junction of ex- ternal and internal.	Junction of ex- ternal and sub- clavian
1							1	+				
2	3	4	2	1	1	1	1	-	Dilatation at termina- tion being formed by several other vessels	+	+	+
3	3	4	1	2	1	1	1	-	Loop just before termi- nation.	+		
4	3	5	2	2	1	2	1	+	Several nodes near ter- mination	+		
5	4	4	1	1	2	1	1	+	Divides at 8th rib and joins at 4th forming loop		+	
6	3	7	2	3	2	1	1	-	Short little vessels go- ing off at 6th and 7th ribs to small nodes to right		+	+
7	3	5	1	2	2	1	1	-	Divides above to nodes and two trunks up to the common termi- nation			+
8	3	4	2	1	1	2	1	+				
9	4	4	1	2	1	2	1	-				
10	2	3	2			1	1					
11	3	4	1	2	1	1	1	-				
12	3	5	2	2	1	1	1	+				

lymph vessels was about four. Some might lie ventral and to the right of the vena cava, while others in the same group lay on a level dorsal to the vein but ventral to the artery. Still others lay dorsal to the artery. These vessels could be easily moved and rolled with the blood vessels and their number and position determined although the same vessel changed its relative position in many cases running more dorsal around the vein or artery or more to right or left.

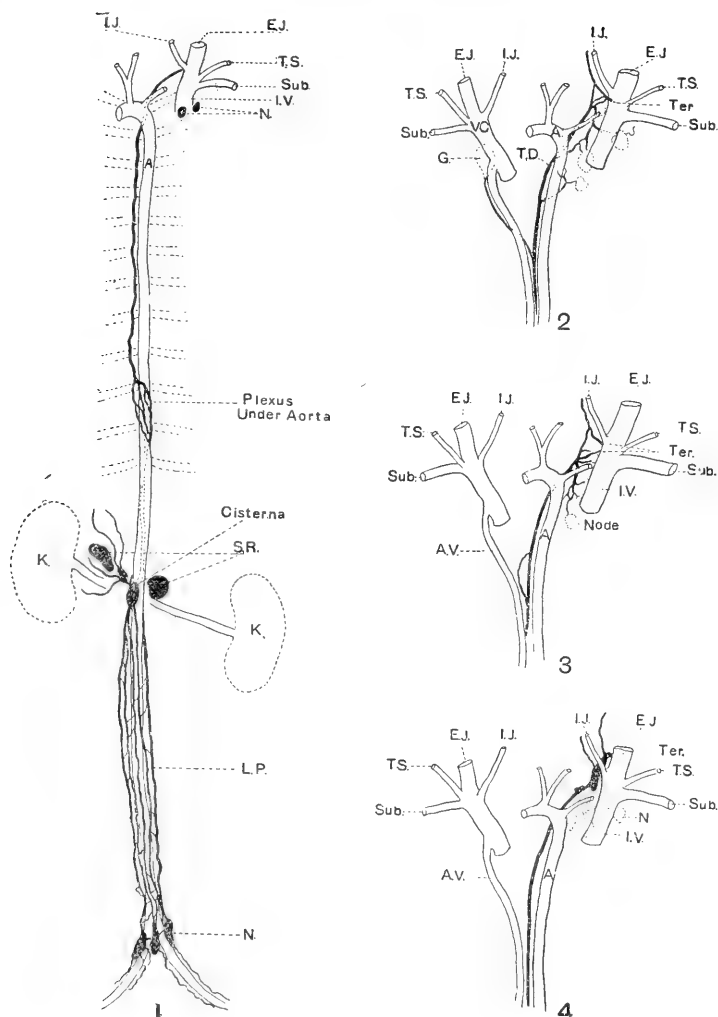
At the level of the left renal vein the group of lymph vessels joined to form two or three vessels which always ran dorsal to the left renal vein and to the left suprarenal gland where they formed the cisterna chyli at the level of the second lumbar vertebra. In this region the mesenteric, diaphragmatic and hepatic lymph trunks join the main lymph vessels. In some cases these join by a common trunk. Generally they flow into a large dilation to the right of the aorta just cranial to the renal veins which is connected to the cisterna chyli by a single trunk or a separate vessel and extend cranially from it and running along the right crus of the diaphragm join the thoracic duct above.

In some cases a trunk from the mesenteric nodes also runs caudally passing ventral to the renal veins and joins the lymph vessels before they reach the cisterna chyli which was almost always distinct. It was a well marked dilatation partly surrounding the aorta, found between the second and third lumbar vertebrae or on the body of the second. It usually lies mainly ventral to the aorta but curves dorsally around the right side. In some cases it was formed by two dilatations one ventral and to the right and the other dorsal and to the left of the aorta which were connected by a large trunk. However, in two cases out of the series of 26 rabbits instead of a distinct cisterna a dense plexus was present. The dilatation into which the vessels from the abdominal viscera terminated was usually located a little cranial and ventral to the cisterna chyli which formed the beginning of the thoracic duct proper. The latter usually left the cisterna by one large trunk which lay dorsal to the aorta and ran in this position through the aortic hiatus in the dia-

phragm up into the thorax always lying to the left and dorsal to the aorta. In some cases a plexus took the place of a single vessel and in others there was a second trunk which started from the ventral part of the cisterna or from the dilatation made by the combination of the visceral lymphatics. This vessel passed to the right side of the aorta along the right crus and at about the 11th intercostal space usually turned dorsally and to the left to join the main trunk. If it did not do this it continued on up on that side of the aorta to a plexus always present at the level of the tenth intercostal space dorsal to the aorta.

The thoracic duct runs up dorsal to the aorta to the level of the 11th rib where it turns to the right and as it crosses always breaks up into a plexus of vessels which lie dorsal to the aorta. The occurrence of this plexus dorsal to the aorta at the level of the 11th rib may be explained by the fact revealed by Lewis ('05) in his work on the lymphatic development in the rabbit. Lewis says that the thoracic duct arises from a plexus of lymphatics surrounding the aorta. Hence it may be that in the rabbit only this part of the plexus persisted into adult life. The duct reappears as a single vessel to the right of the aorta at the level of the tenth rib or a little cranially to it. The dorsal plexus which is made up of from 3 to 5 vessels is always present. As the duct extends cranially on the right side of the aorta and dorsal to it, it lies ventral to the azygos vein. It thus extends to the level of the third rib where it comes to lie on the longus colli muscle. At the level of the second rib it begins to cross to the left and passes dorsal to the aortic arch as one vessel. It then runs up in the interval between the innominate and the left subclavian arteries to its termination into the left external jugular vein at the angle of junction of the internal jugular where the presence of nodes is very common.

Hence forming the main lymph channels of the trunk we find, as shown in figure 1, a group of vessels running up through the abdomen around the large blood vessels and with the visceral vessel forming a cisterna at the level of the second lumbar vertebra. From this on extends one duct which lies to left of aorta to the tenth intercostal space where it crosses forming a plexus dor-



ABBREVIATIONS

I.J., internal jugular
E.J., external jugular
T.S., transverse scapular vein
Sub., subclavian vein
A., aorta
T.D., thoracic duct
G., gland

A.V., azygos vein
I.V., innominate vein
N., node
S.R., suprarenal gland
L.P., lumbar gland
K., kidney

sal to the aorta. From this plexus a single trunk extends cranially ventral to the azygos vein and to the right of the aorta to the second rib. At this level it crosses to the left again dorsal to the arch of the aorta and extends on up to its termination into the veins to the left as a single vessel.

Variations from the type of duct just described were very few. In 2 out of the 26 cases nos. 13 and 18 I found a plexus in place of a cisterna chyli. The plexus in each case surrounded the aorta and was longer than the average cisterna found in the other cases. In case of one of these the plexus extended on cranially as a narrow plexus on the dorsal side of the aorta up to the plexus at the 10th intercostal space which was described before as being always present.

In 9 cases out of the 26 small vessels were seen in the lower thoracic region running laterally at right angles from the duct to the right and dorsalwards, some lying ventral to the azygos, while others were dorsal. The usual place for these was at the plexus found dorsal to the aorta where the duct crosses over, or slightly above in the eighth or ninth intercostal space. There were never more than four and in most of the cases only one or two vessels. They came off at irregular intervals some close together while others were in the intercostal spaces. In 5 out of these 9 cases they were traced to small nodes found in the intercostal spaces near the sympathetic ganglia. In the other 4 the vessels were small and extended dorsally but it was not possible to determine distinctly where they went and in no case could I trace these to the azygos vein as reported by Witzer ('34) in a human subject and by Boddaert ('99) in the rabbit. Indeed in only one case a vessel was given off from the duct to the left at the level of the twelfth rib. It terminated in a small node. Another variation found was the presence of two ducts in two cases. In each of these instances two ducts came off from the retro-aortic plexus. In one case it came off from the plexus at the tenth intercostal space and ran to the left of the aorta to its termination. This vessel passed through a large group of nodes in relation to the left vena cava, from which it received tributaries and continued on up running dorsal to the

left subclavian artery and also dorsal to the main thoracic duct and terminated cranially to it at the junction of the external and internal jugular veins. The main duct on the other hand terminated at the innominate vein farther down and received a communicating vessel from the accessory duct as it passed ventral to it.

In the other specimen the accessory duct at first turned to the right of the main duct for a length of two centimeters, then curved back to the left and dorsal to the main duct and aorta and ran up on the left side receiving vessels from nodes around the left vena cava and terminating caudal to the main duct in the innominate vein.

Another variation was the presence of a vessel extending from the thoracic duct over to a node of variable size which usually lay under the right innominate vein. This occurred in 10 out of the 26 cases and hence was fairly frequent. The vessel usually came off from the duct at about the level of the third rib and ran cranially and to the right on the longus colli muscle to the node which lay at the first rib. In one case two vessels came from or joined this node one on each side of the right vena cava.

The presence of small communicating loops was noticed in many cases. These were formed by a small vessel branching off from the thoracic duct and then running cranially a little distance and again joining the duct. This occurred in about 9 cases at a region between the retro-aortic plexus and the aortic arch. In some instances the loop was longer than in others. In one instance it was about 4 cm. in length but others were shorter even as short as 5 mm. Most of these loops occurred where the duct lay on the longus colli muscle just before passing dorsally to the arch. In some cases small vessels were given off from these loops to surrounding nodes and in one case also ventrally to the aorta.

Figure 2 illustrates the termination of the thoracic duct in rabbit no. 12. It has a single termination at the junction of the left internal with the left external jugular. It divides and is joined by vessels from surrounding nodes which are always present and finally empties by a single trunk into the large vein.

This is the type of termination found in 16 out of the 26 rabbits. In some instances instead of dividing just before reaching the termination the duct formed a large dilation and in others it passed directly through nodes lying in this region. In every case the lymph nodes especially those on the left side and around the three great vessels here illustrated, were completely injected. In some of the cases vessels could be seen coming from the nodes and joining the duct just before it terminated. These were most likely efferent vessels which must have been injected back from the duct. In one case a node fully 2 cm. in length lying dorsal to the left innominate vein was completely filled by the black injection fluid. This indicates that the valves in these vessels were either incompetent or had broken down.

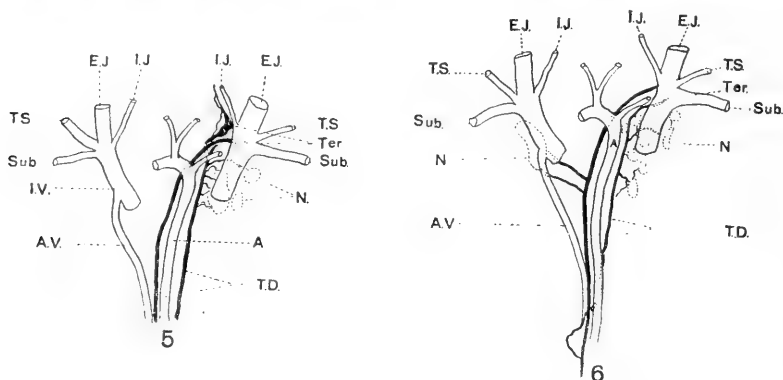
In figure 3 I have illustrated the type of termination found in 5 out of the 26 cases. The termination is double. One trunk terminates at the junction of the internal with the external jugular and the other joins the common jugular vein further caudally. In two of the cases it was hard to determine whether the most cranial duct terminated in the internal jugular itself before the junction was reached or whether it terminated at the junction of the jugulars. In all the five cases it divided after it had emerged from the dorsal side of the aortic arch. Injected nodes were also seen in this region. The termination of the second duct varied a little in its position being close to the main duct in some cases while in others it was a considerable distance caudally.

Figure 4 represents the type of termination found in three out of the 26 rabbits. The termination was single in these cases and was found at the external jugular cranial to its junction with the internal jugular as a rule and usually running dorsal to the internal jugular in its upward course. In two of these cases a large dilation was found just before the duct terminated. From this dilation a lymph vessel always ran up the neck along with the internal jugular vein.

Figure 5 represents the mode of termination of one of the double ducts found in this series. The left duct came off from the plexus dorsal to the aorta at the tenth intercostal space and ran

up on the left side. These ducts empty separately. The one at the junction of the jugulars and the other into the common jugular. After passing through a group of nodes the left duct passes on up dorsal to the subclavian artery and to the main duct to which it sends a communicating vessel and then forms a large dilation which joins the junction of the jugulars. The main duct takes the usual course and empties into the common jugular below.

Figure 6 represents the other case of a double duct found in this series. The accessory duct branched off from the main duct at the eighth rib extending to the right and gradually



Figs. 5 and 6 Modes of termination of the thoracic duct.

curving back to the left, dorsal to the main duct and the aorta. It then passed through a group of nodes to the left and terminated in the innominate vein while the main duct followed the usual course and terminated at the junction of the external jugular with the subclavian vein.

These five types here described include all of my series. McC lure and Silvester ('09) have illustrated the termination of the duct in four rabbits and their results are very similar to those obtained by me. In two out of their four cases the termination was single and into the junction of the internal and external jugular veins. This was the predominating place of termination in my series. In their rabbits they found two with double ducts which are similar to those cases in which I found two ducts.

Upon comparing the termination of the thoracic duct in the rabbit with other animals we find a considerable difference. In monkeys as found by McClure and Silvester the most general termination was a single trunk terminating in the jugulo-subclavian junction. According to McClure and Silvester the two jugulars and the subclavian veins in monkeys usually join at a single point. According to Ellenberger and Baum the termination in the dog is usually double. One duct joining the junction of the left internal and external jugulars but the other the external jugular, a condition very similar to the rabbit. The relative size of the position and junctions of the veins is also very much like that found in the rabbit. But according to McClure and Silvester and of Sisson ('11) and of Chauveau ('10) the thoracic duct of the cat usually terminates in the external jugular by two trunks. According to Sisson the thoracic duct in the horse takes quite a tortuous course and usually terminates in one trunk which joins the anterior vena cava just behind the angle of junction of the two left jugular veins. The duct in the horse is said to develop from a plexus and hence varies considerably. Often there are two ducts.

In the ox the duct is said to be very variable, hardly ever being single but often double or plexiform. Its termination also varies being single or double but it usually empties into the junction of the jugular and brachial veins.

Parsons and Sargent ('09) who have investigated about 40 cases in man found that the duct in 75 per cent of the cases terminated as a single trunk. This trunk joins the internal jugular below the left flap of the valve where the jugular joins the left subclavian vein. In only 7.5 per cent of the cases was the termination at the junction. In 22 per cent of the cases the termination was by two trunks into the last centimeter of the left internal jugular vein. In many of the cases the duct bifurcated and then reunited sometimes only in the wall of the vein. In 2 cases out of the 40 multiple terminations were met with but never more than four. Clattenberg found the termination in the guinea pig usually single and into the innominate vein just at the junction of it with the internal jugular.

Nevertheless the duct is usually double up to within a very short distance of its termination. The arrangement of the great vessels is quite different in the guinea pig than in the rabbit.

According to Job ('15) the left duct of the rat empties into the jugulo-subclavian junction but is said to carry lymph from only about half of the body.

Upon comparing my results with those found in other animals I find that there is a considerable difference in some respects while in others the differences are slight. In the horse, it is said, the cisterna chyli is about 10 cm. in length, ampullated and lies dorsal to the aorta and to the right opposite the first and second lumbar vertebrae. It is always very definite and is formed by two trunks from the viscera and one or two lumbar trunks. It seems that the lumbar trunks are not regular vessels but contain nodes of considerable size and are also joined at intervals by lymph vessels. In the rabbit, on the other hand, a group of parallel vessels always run through the abdomen. These are never interrupted by the presence of nodes along their course. They combine and with those from the viscera and the diaphragm form a cisterna chyli which is not always very definite. From the cisterna in the horse the duct runs up through the aortic hiatus and forward on the right of the median plane between the azygos vein and the aorta. It is generally single and extends up to the sixth or seventh thoracic vertebra where it crosses ventral to the oesophagus and then runs to the left of the aorta to its termination.

In case of the rabbit the duct first lies to the left of the aorta and as it passes through the aortic hiatus dorsal to it. It then crosses to the right as described before at the tenth intercostal space and as it does so breaks up into the plexus dorsal to the aorta. This plexus, it seems, is never found in the horse. Crani-ally from this plexus the duct in the rabbit, lies in the same position as in the horse but crosses to the left, again much higher up and dorsal to the oesophagus.

Comparing the duct in the rabbit with that in the ox we find some interesting variations. Instead of the group of lumbar vessels found in the rabbit there is usually only one large duct

extending up from the lumbar glands and joining the large gastrointestinal trunk caudal to the renal veins dorsal to which it passes to form a small cisterna. From the cisterna in the ox there may be one or two ducts which may pass through the aortic hiatus or there may be several anastomosing ducts. There hardly ever is only one duct as is typical in the horse. These ducts extend up and the one on the right crosses over at varying heights usually joining the left and terminating as one. In the ox the duct is more variable than in the rabbit.

Comparing the thoracic duct of the human being with that in the rabbit we note few differences. In the abdominal region of man we have two trunks which extend up from the pelvis and lower extremities and the lumbar glands which lie separated by the large abdominal vessels in the midline. These vessels pass dorsal to the renal veins and are joined by a large intestinal trunk which also passes dorsal to these veins and by two descending trunks from the thorax which pass through the ventral part of the aortic hiatus. All of these vessels go to form the cisterna chyli which is about 5 to 6 cm. in length and lies to the right between the aorta and the lower part of the vena azygos, posterior to the right crus of the diaphragm and opposite the first and second lumbar vertebrae. According to Davis ('15) a definite cisterna is only present in 50 per cent of cases in man. From the cisterna the human duct is single and extends cranially through the aortic hiatus. Lying dorsal and to the right of the aorta but ventral and to the left of the vena azygos, the duct terminates singly in 89 per cent of the cases into the left subclavian vein, and in 22 per cent of the cases into the junction of the jugular and subclavian.

In the rabbit, on the other hand, we find a group of 4-6 anastomosing lumbar vessels closely grouped around the large blood vessels with a relatively smaller and indistinct cisterna. From the cisterna in the human being one duct usually extends cranially through the aortic hiatus, lying dorsal and to the right of the aorta but ventral and to the left of the vena azygos. The vessel runs cranially in this position to about the fifth thoracic vertebra. Here it crosses over to the left dorsal to the aorta

and the oesophagus and from there extends out of the thorax on the left side of the aorta.

In the rabbit the duct crosses the vertebral column, once at the tenth intercostal space in the form of a plexus and once above at the second vertebra. This crossing is very similar to that in the man but a plexus is never present in the latter where the duct crosses the vertebral column. Of the many variations which have been found in the human subject none known to me have the characteristics found typical in the rabbit.

Comparing the cisterna of the rabbit with that of the cat we find that in the cat the cisterna is formed by the intestinal trunks and two or three lumbar trunks. It is definite and lies opposite the second lumbar vertebra. From the cisterna the thoracic duct which may be looped extends up as a single duct but always lies to the left and terminates in the left external jugular vein.

In *Bos taurus* according to Baum, the cisterna is not very large. It is formed by two lumbar trunks which join the visceral trunks caudal to the renal vessels. These vessels join and form one large trunk which runs dorsal to the renal vessels to the cisterna opposite the second lumbar vertebra. From the cisterna the duct runs to the right of the aorta to the fifth thoracic vertebra. Here it crosses dorsal to the aorta and extends up on the left and terminates into the left external jugular vein.

In the dog the cisterna is relatively very much larger than in the rabbit. It is long and ovoid and even extends into the thoracic cavity between the crura of the diaphragm. From the cisterna the duct is usually single, extends up on the right and then crosses over at about the fourth or fifth thoracic vertebra to terminate in the left external jugular vein.

In the guinea pig according to Clattenberg a definite cisterna is not always present. The abdominal vessels are plexiform extending up in the midline. At the level of the renal vessels a number of nodes are usually found in which the plexus is lost. From these nodes extend vessels which form a dilation opposite the first and second lumbar vertebrae dorsal to the blood vessels.

Cranially from the cisterna another plexus of vessels may leave the nodes just mentioned and pass directly into the plexus above the cisterna. This plexus is usually wide and extends up into the thoracic cavity to the level of the eighth rib. From the eighth rib on up Clattenberg almost always found two separate ducts one on each side of the aorta. The one on the right side crosses over at the third rib and forms another small plexus with the left. Cranially from here a single duct which terminates at the junction of the innominate and the internal jugular veins on the left is found. This form of duct is much like the embryonic plexus found in most mammals.

In conclusion I wish to thank Professor Meyer for the help he gave me in the course of this investigation.

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SOME RESULTS AND POSSIBILITIES OF EARLY EMBRYONIC CASTRATION

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SIX FIGURES (FOUR PLATES)

The works of Eigenmann (7, 8), Hoffmann (11), Nussbaum (13), and Beard (3) have supported the proposition that the germ-cells in several vertebrates are precociously segregated, and that their locus of origin is not necessarily the 'germinal epithelium' or even its immediate region. The classic case of early segregation of the germ-tract of a vertebrate is that described by Eigenmann, who was able to trace the lineage of the sex cells of *Micrometrus aggregatus* probably as far back as the fifth cleavage; this is the farthest that any vertebrate germ-cell has ever been traced. Hoffmann discovered the existence of primitive ova in the mesenchyme of a number of bird embryos at a time prior to the establishment of germinal epithelia in those embryos. For a review of the works of Beard (3), Nussbaum (13), Rubaschkin (14), von Berenberg-Gossler, Allen (4, 1, 2) and Fuss (9), and others, the reader is referred to the introductory discussion by Swift (18). For a discussion of the segregation of the germ-cells in the invertebrates, Hegner's 'Germ-Cell Cycle' may be profitably consulted.

The work of Hoffmann and others seems to be borne out by the highly interesting work of Swift (18). The latter has not only strengthened our belief in the extra-regional origin of the sex-cells in the chick, but he has offered a solution of the question why the primordial germ-cells of the chick had never been found prior to the twenty-two somite stage. He maintains that the sex-cells originate in a crescent-shaped area of the extra-embryonic blastoderm anterior to the body-axis at the line of

demarcation between the areas pellucida and opaca (see his figure 15); that these primitive sex cells reach the gonad partly by their own wandering, but principally by way of the blood-stream which transports them either to the gonad where they continue to develop, or to some other region where they soon degenerate. He makes the highly interesting suggestion that the large wandering cells which Dantschakoff (5) found to degenerate in the blood-stream following the twenty-two somite stage are really germ-cells which failed to become incorporated into the gonad.

At a very short time subsequent to the publication of the work of Swift, Professor McClure in 1914 suggested to one of his students that this work of Swift's could be verified or disproved by the early excision of this crescent-shaped area described by that author, provided the operation be done at a time before the germ-cells have wandered away from this area of proliferation. The work was attempted but no results were obtained. Since then the writer has spent a great deal of time trying to develop a technique such that the embryos might be maintained despite the extremely low viability of the experimental material. In this work, some few thousand chick embryos have been sacrificed. During the first year of the work it was possible to rear a few embryos to the seventy-two hour stage; later than this, two embryos survived to the age of five days. These were, however, discarded on the assumption that they were unfit for critical study owing to the fact that they had died a short time before they were preserved. This is explained by the fact that such embryos were always allowed to develop as far as they would. It was not always possible to find them in the act of dying. It was found, however, that in normal individuals which were bled to death and then allowed to stand many hours before preservation that the germ cells were quite easily distinguishable in these corresponding stages; material which has been dead a few hours is of some value, although it is unfavorable for the distinguishing of such fine structures as mitochondria. I have tried to rear the embryos to the more advanced ages for the reason that my chief interest lies not in

the confirmation or disproof of the work of Swift, but rather in determining the later effects of this extraordinary early castration. It occurred to the writer that if this crescent-shaped area is the seat of the keimbahn, its removal would afford a means of the earliest castration ever yet performed on a vertebrate; it would give an opportunity for studying in pure culture the interstitial cells of the gonad—particularly their supposed effect on the secondary sexual characters. If, as castration of animals subsequent to hatching or to birth has shown, the secretion of the gonad profoundly affects the secondary sexual characters, it is reasonable to suppose that it might affect some of those sexual characters which are usually considered as primary—such as the persistence or degeneration of the Wolffian and the Müllerian duct. If the latter be true, early removal of the primordial sex cells might possibly cause the retention of the original ground-plan of the urogenital system, namely the simultaneous existence of both of Wolffian and Müllerian ducts (or at least one of the latter), if however the development and normal functioning of the interstitial cells were in no way impaired by the absence of sex cells, their secretions would cause the alteration of the ground-plan if this were their normal function. But on the other hand, if the gonad were incapable of producing a secretion in the absence of sex-cells, opportunity would be afforded of studying such response or irregularities as might be observed in the development of the other ductless glands.

One castrated embryo was recently reared to an age at which one or the other set of genital ducts should have been eliminated. The ground-plan was found to be persistent when the embryo was dissected, but there is a possibility that an arrest of development due to operation played a part in the result.

An ideal way to confirm the work of Swift would be to transplant a 'crescent' from an embryo of one pure breed to the mesenchyme of an embryo of a different breed but of the same sex. If such an embryo could be reared to the adult stage its germ-cells could be tested by breeding. I have been able to rear an embryo into which a crescent was transplanted, to the age of twenty-one days when it died the day on which it should

normally have hatched. Dissection of this embryo showed it to be a male.

Artificial hermaphroditism would be produced in fifty per cent of such transplantations as this just described.

These are some of the problems raised by Professor McClure's suggestion, and which are now in progress of investigation.

Some results of this work at its present status may be of interest. I shall describe at this time the results of some experiments by means of which I have convinced myself, at least, that the work of Swift has given us a more correct and more ultimate solution of the origin of the germ-cells than any other yet given in case of the chick; that his work is of the very greatest importance owing to the experimental possibilities which have arisen from it. The description consists of a comparison of a few individuals in which castration was complete or at least very nearly so, with normal individuals at corresponding stages.

According to Swift, most of the germ-cells have assembled at the base of the mesentery back of the twenty-second somite when the embryo has reached the stage of thirty-three somites. In this position they remain until the germinal epithelium begins to thicken. They then migrate into the gonad. This is seen to be in process in my figure 1. In most of the adjoining sections, primitive ova are being incorporated into this thickened epithelium. The sections of this embryo are only five micra in thickness, yet it is impossible to find a section in the gonad-region which fails to exhibit sex cells. Some of these cells are found far ventrally in the mesentery. The figure shows the slight protuberance of the right gonad; the region illustrated is very similar in location to that of figure 3 and the small rectangle in the keyplate for the latter. In the left upper corner of figure 1, a few erythrocytes have been inserted; they are drawn to the same scale as the rest of the figure. In every case, erythrocytes were used for comparison with the size of the sex-cells.

Figures 2 and 3 are from a section of the left gonad of an embryo slightly older than that from which the preceding figure was made. The gonads of the two embryos were, however, about equally prominent. The germinal crescent of this em-

bryo was excised at a time previous to the establishment of any intersomitic grooves and at which the neural folds of the brain were first indicated. The embryo was killed at the age of ninety-four hours. In this section it will be seen that the germinal epithelium is very little thickened. As in all other sections of this embryo, germ-cells are entirely absent. Only a few cells in the mesenchyme approach in size the erythrocytes shown in the figure; these are not germ-cells. None can be found which exceeds the size of the erythrocytes to any such extent as do the germ-cells which are invariably present in sections of the gonads and mesentery of embryos of this age, and which can be most readily identified, no matter what may be the technique of fixation or staining employed.

Figure 4 is a section through the left gonad of a normal chick embryo at the age of one hundred and nine hours. This excellent series was prepared about fifteen years ago by Prof. A. M. Miller. The gonad protrudes considerably into the coelom. Among the very compact interstitial cells are numerous lightly staining germ-cells of large size. The mesothelium of the mesentery adjacent to the gonad is very greatly thickened, darkly staining, and as in all normal individuals of this age, contains numerous ova which cause local protrusions into the coelom. It will be noted that these germ-cells which have been incorporated into the mesothelium are slightly larger than those which have reached the gonad. Lying in the mesenchyme of the mesentery are large germ-cells. These can usually be found in any section of a mesentery at this age. Pictures quite similar to this are readily obtained in embryos one hundred and twenty-five hours old except that the sex cells found in the gonad are relatively somewhat smaller. It will generally be found that this size-relationship obtains in normal embryos. The germ-cells which remain in the mesenchyme are usually the largest. It may be that those in the gonad undergo more rapid multiplication. A few erythrocytes sketched indiscriminately from the dorsal aorta are inserted for comparison of size.

Germ-cells can be found in the mesenchyme of the mesentery as late as one hundred and seventy-five hours. This is the oldest material which I have examined for this particular point.

In these older stages, many instances will be found in which nuclear division in such germ-cells has not been accompanied by cytoplasmic division, so that a large multinuclear cell results, having a diameter about equal to that of the mononuclear germ-cells of the thirty-three somite stage. Such a condition is shown in figure 5. A very interesting condition is found here; it will be noted that a large binucleate cell-body projects from the mesenteric surface and touches the gonad from which there is likewise a slight protrusion and on which there is also an interruption of the mesothelium. These are undoubtedly germ-cell nuclei. If this is a case of chemotaxis it is a very remarkable one. It is entirely possible that such proliferations from the coelomic wall might sometimes be misinterpreted as giant blood-cells. In the gonad will be seen a germ-cell which is smaller than those lying in the mesenchyme.

We may now consider the conditions which obtain in a castrated embryo five days old. The operation was performed just prior to the establishment of the first intersomitic groove. If castration was not complete, it was very nearly so. In this embryo I have found about five cells which are considerably larger than the average erythrocyte, and several which are a little larger. I am convinced however, that these are not germ-cells. If one examines Swift's (18) figure 6, for instance, he will find at least two mesenchyme cells in the left upper corner of the figure which are larger than the erythrocyte in the small endothelial cavity at the lower right corner. There would be little danger of confusing these with the large germ-cells present in the figure. I have made no attempt to diagnose germ-cells on the basis of the form of the mitochondria, since there is not perfect agreement on the question whether the germ-cell mitochondria of the chick are characteristic. See Rubaschkin and Tschaschin (14, 15, 16, 17, 19) and Swift (18) p. 496.

The conditions in this five-day castrated embryo are illustrated in figure 6. The section passes through the right gonad. The mesothelium of the mesentery consists of a single layer of cells. In all normal individuals at this age this mesothelium near the gonad is found to be greatly thickened, stains darkly and contains sex-cells. There are no germ-cells in the mesenchyme of

the mesentery, whereas they are invariably present in the normal individual. The gonad contains only interstitial cells, so far as those who have examined the material have been able to detect. The gonad tissue is greatly vacuolated, while in normal embryos even at younger stages the gonad is quite compact and remains so even through rough histological treatment. The interstitial tissue displays solid darkly staining lines of intercellular substance bordered by interstitial cells the arrangement of which gives a foliage-like appearance. In some cases the plane of section passes through an interstitial cell which projects into a large vacuole. When the diameter of such a vacuole is about that of a germ-cell the picture is somewhat similar to that which a germ-cell in a normal gonad would present if the cytoplasm were dissolved out. Professor E. G. Conklin has examined this material and has pronounced the fixation to be sufficiently perfect that germ-cells should be easily distinguished if they were present. If any are present they are certainly very few.

In the foregoing account, the term 'stroma tissue' might well have been used instead of the term 'interstitial tissue.'

It seems reasonable to believe that the abnormal conditions recorded and the early removal of the crescent-shaped piece of blastoderm are causally related. In conclusion I wish to thank Professors C. F. W. McClure and E. G. Conklin for the aid which has made this work possible.

SUMMARY

1. The extra-regional origin of the germ-cells of the chick may be regarded as highly probable.

2. The early location of the germ-tract on the yolk-sac gives opportunity for the earliest castration yet performed on a vertebrate. It makes possible an analysis of the functional activity of the sex-cells and the interstitial cells in the production of internal secretions, the effects of the latter on the sexual characters and on other characters. It makes possible the production of artificial hermaphroditism, provided the germ-cells of one crescent can be made to enter the gonad of an embryo of the opposite sex. The work of Swift is of very great importance.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

- 1 Section through the right gonad of a normal chick embryo ninety hours old. Picro-acetic fixation. Iron haematoxylin followed by methyl blue-eosin. $\times 800$. In most adjoining sections, the germ cells have entered the germinal epithelium. This section is not typical, but it illustrates an interesting condition.
- 2 Low-power sketch of the section from which figure 3 is made. The rectangle to the left of the mesentery indicates roughly the regions from which figure 3 is taken. *G. eph.*, germinal epithelium, *Mst.*, mesentery; *Pr.O.*, primitive ova.

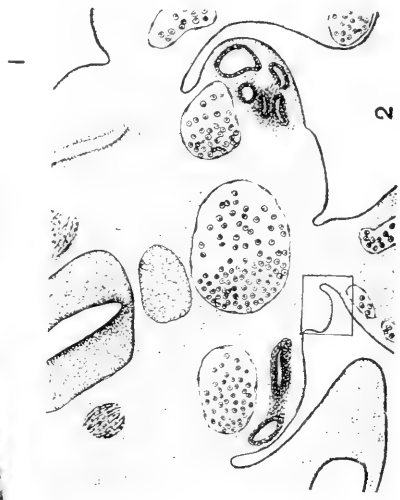
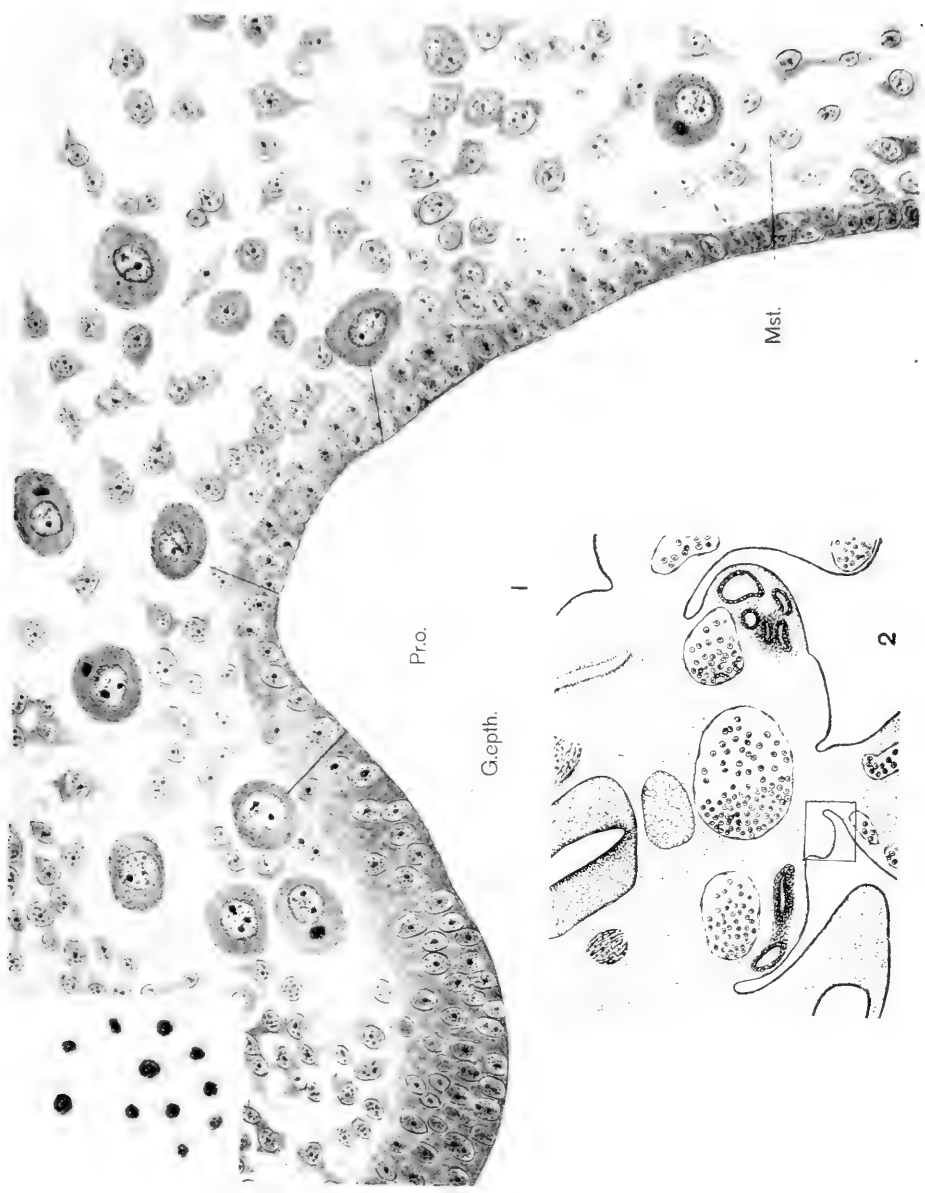


PLATE 2

EXPLANATION OF FIGURES

3 Section through the left gonad of a ninety-four hour chick embryo which was castrated prior to the formation of any intersomitic grooves. Note the absence of germ-cells and the thinness of the germinal epithelium. The section is viewed from its *posterior* surface. Benda's fixation followed by Meves' iron-haematoxylin stain. $\times 800$. *Endth.*, endothelium; *Erth.*, erythrocytes; *G. eph.*, germinal epithelium. *Msth.*, mesothelium.

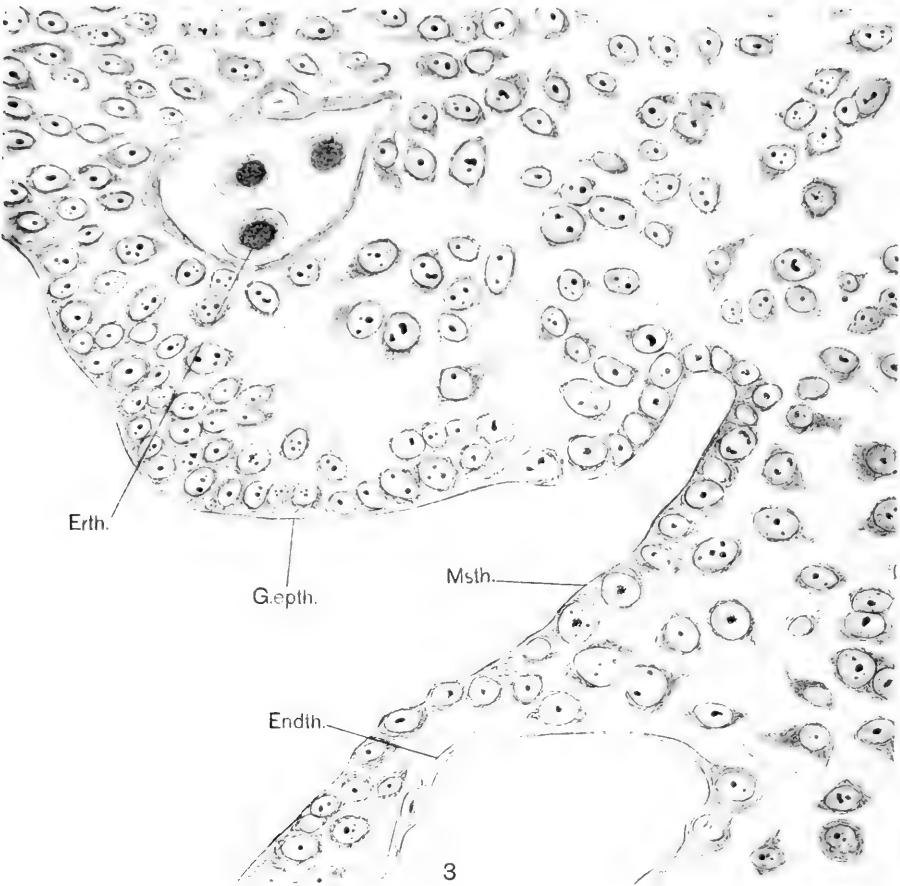


PLATE 3

EXPLANATION OF FIGURES

4 Section through the left gonad of a normal chick one hundred and nine hours old, showing germ-cells in the gonad, and in the mesothelium and the mesenchyme of the mesentery. Zenker fixation. Delafield's haematoxylin and orange-G. Princeton Embryological Collection, series no. 46. $\times 800$.

5 Section through the left gonad of a normal chick one hundred and sixty-two hours old, showing primitive ova in the gonad and in the mesentery. Fixation and stain as in figure 4. Princeton Embryological Collection, series no. 41. $\times 800$. *Coel.*, coelom; *Gon.*, gonad; *Mst.*, mesentery; *Msth.*, mesothelium; *Pr.O.*, primitive ova.

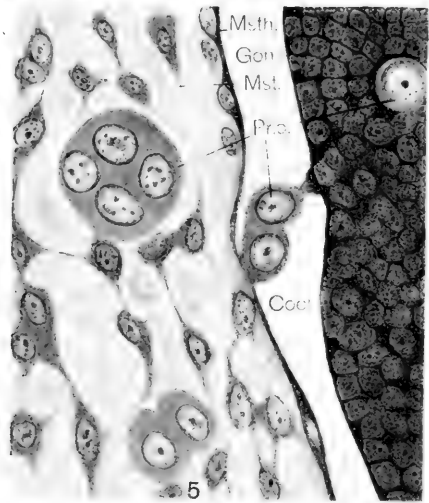
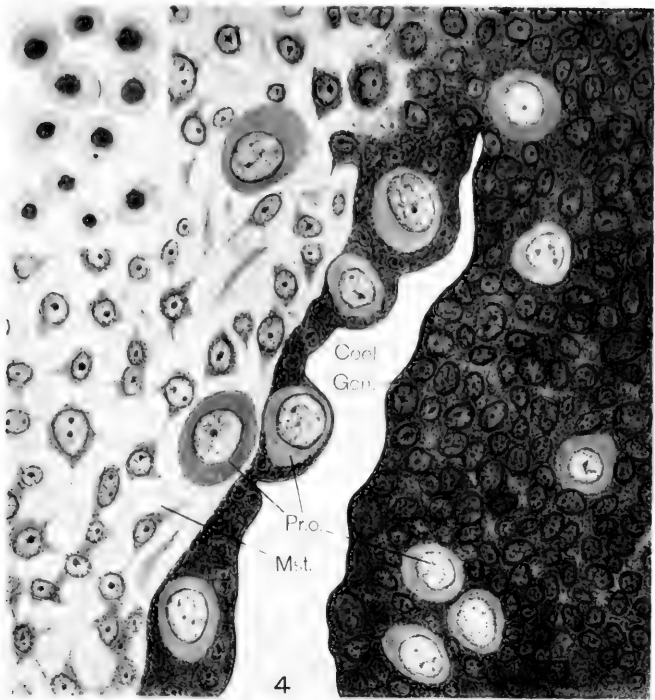
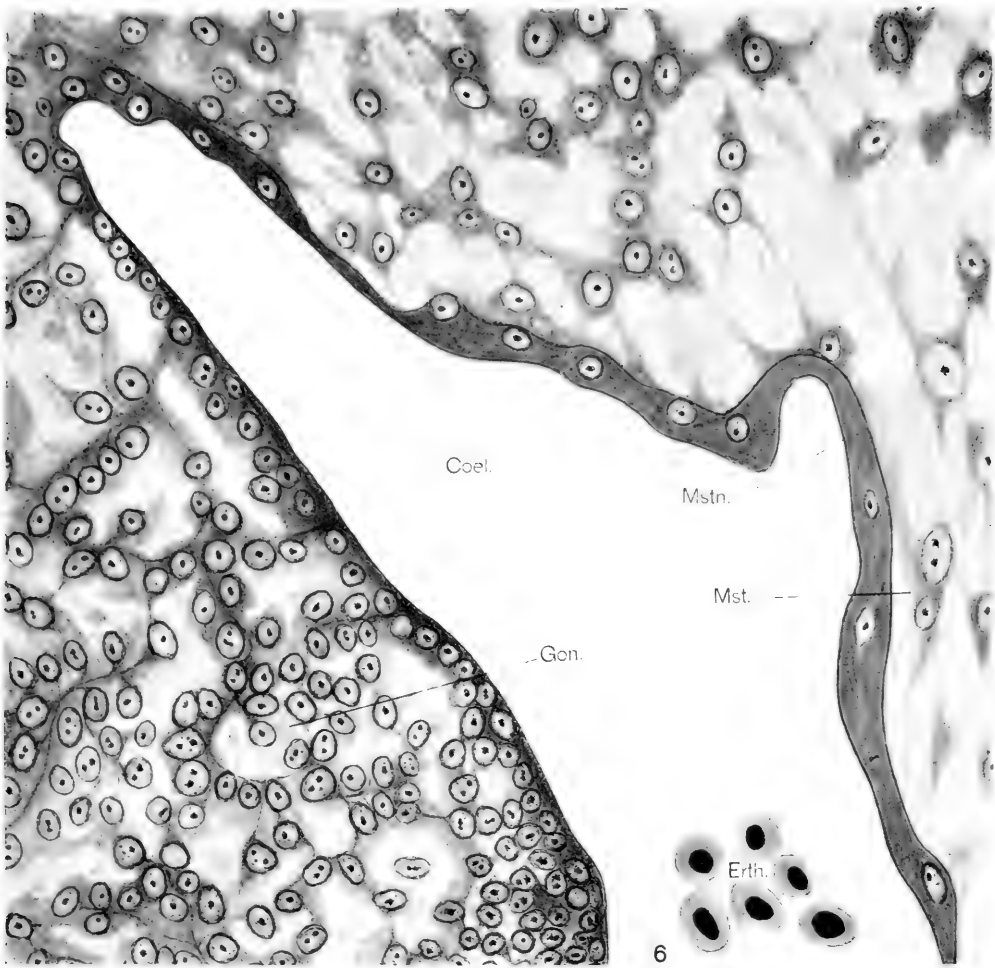


PLATE 4

EXPLANATION OF FIGURE

6 Section through the right gonad of a one-hundred-and-twenty-hour chick which was castrated at a time prior to the establishment of the first inter-somitic groove. The mesentery, the mesothelium and the gonad are devoid of sex cells. Note the peculiar foliage-like appearance of the gonad tissue. A few erythrocytes drawn to scale were inserted into the lower part of the figure. Bouin's fixation followed by iron-haematoxylin and eosin. The left gonad would have afforded a still better comparison with figure 4. The right one was chosen for the reason that the stroma-tissue was less dense than in the left. Both gonads are almost the same size. The mesothelium near the left gonad is very little thicker than that on the right. The left gonad contains no germ cells. $\times 800$. *Coel.*, coelom; *Erth.*, erythrocytes; *Gon.*, gonad; *Mst.*, mesentery; *Msth.*, mesothelium of mesentery.





THE RELATION OF AGE TO FERTILITY IN THE RAT

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THREE FIGURES

Data have recently been obtained that show the complete breeding history of a considerable number of female rats. An analysis of these data with reference to the question of fertility and its relation to age seems desirable, since literature dealing with litter size in rodents (bibliography in 'The Rat,' Donaldson, '15) gives very little information on this point and fails to record the entire litter production of even one pair of animals.

The breeding records of seventy-six females that produced a total of 585 litters are used in this study. The majority of the females (50) were piebald or 'hooded' rats; the rest were either 'extracted' albinos (15) or 'extracted' grays (11). All three strains were derived from the F_2 generation of a cross between the wild Norway rat (*Mus norvegicus*) and the domesticated albino (*Mus norvegicus albinus*). Mention is made of the kind of rats used merely as a matter of reference. The conclusions drawn from the results are doubtless applicable also to other strains of rats.

All of the females lived to be at least sixteen months of age, the oldest dying at the age of twenty-three months. Under the conditions existing in the animal colony of The Wistar Institute a rat is usually in its prime at the age of seven or eight months, and after reaching twelve months of age it is classed as 'old.' Very few individuals live for more than twenty months, although all animals are kept under environmental conditions that are seemingly well suited to their needs. The relatively early death of the animals is due, in part at least, to the fact

that seasonal changes in temperature in the region of Philadelphia render old animals very susceptible to pneumonia, the disease that invariably proves fatal to a rat of any age. In a more equable climate, like that of California, rats have been kept in good physical condition until they were four years old (Slonaker, '12).

In the rat the menopause usually appears at the age of fifteen to eighteen months (Donaldson, '15, p. 21). Data covering the litter production during the first sixteen months of life, therefore, may be assumed to show the actual fertility of the great majority of females. The word 'fertility' is here used as defined by Pearl and Surface ('09) to designate: "the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring." Fertility, according to this definition, is a much more comprehensive term than fecundity with which it is often confused. The latter, as suggested by Pearl and Surface should properly be used to signify only "the innate potential reproductive capacity of the individual organism as denoted by its ability to form and separate from the mature body germ cells."

Litter data for the three strains of rats are shown in table 1.

TABLE 1
Showing litter data for the three series of rats

	NUMBER OF BREEDING FEMALES	NUMBER OF LIT- TERS CAST	A V E R A G E NUMBER OF YOUNG PER LITTER	TOTAL NUMBER OF YOUNG	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
Piebald series.....	50	406	6.9	2798	1447	1351	107.1
Extracted albinos.....	15	88	6.2	548	279	269	103.7
Extracted grays.....	11	91	6.7	609	310	299	103.6
	76	585	6.7	3955	2036	1919	106.1

As table 1 shows, the corresponding records for the three series are very similar. The differences in regard to litter size and to the relative proportion of the sexes that are found are well within the limits of the variation that is always to be ex-

pected when the number of records is comparatively small. For further analysis, therefore, the data for the three strains have been combined. The entire series of records, arranged according to the age of the mothers when the litters were cast, is given in table 2.

The 'mean age of the females,' as given in the first column of table 2, is the median point of a thirty day period in the life of

TABLE 2

Showing the number of litters in the combined series, together with the sex ratios and the coefficients of variation for litter size. Data arranged according to the age of the females when the litters were cast

MEAN AGE OF FEMALES IN DAYS	NUMBER OF LITTERS CAST	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUM- BER OF IN- DIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALE	COEFFI- CIENTS OF VARIATIONS FOR LITTER SIZE
90	38	6.9	264	126	138	91.3	34.9
120	49	7.9	389	207	182	113.7	32.3
150	57	7.6	433	215	218	98.6	26.5
180	60	7.8	472	252	220	114.5	31.5
210	61	7.7	471	243	228	106.5	25.2
240	46	7.3	337	155	182	85.1	36.8
270	44	7.0	314	163	151	107.9	36.2
300	49	7.4	363	187	176	106.2	35.7
330	41	6.0	246	138	108	127.7	37.8
360	31	6.1	191	97	94	103.1	36.5
390	35	5.1	179	104	75	138.6	51.2
420	26	4.5	118	64	54	118.5	41.8
450	18	4.3	79	35	44	79.5	51.5
480	13	3.2	42	23	19	121.0	73.3
510	10	3.4	34	17	17	100.0	36.3
540	6	3.6	22	9	13	69.2	47.7
570	1	1.0	1	1			
	585	6.7	3,955	2,036	1,919	106.1	38.0

each animal, except in the two cases noted below. For example, the mean age '120 days' includes the records for all litters produced by females that were from 105 to 135 days of age when parturition occurred. The ninety day group is one exception to the above rule; it comprises litter records for a twenty day period only, as the youngest mother in the series was eighty-six days old when her first litter was cast. One female gave birth to a litter of one when she was 594 days old. For the

sake of uniformity this record is put under the mean age '570 days' which is thus extended to include a period of forty-four days.

The majority of female rats that are in good physical condition cast their first litters when they are about three months old. Thirty-eight of the seventy-six breeding females bore young before they were 105 days old; all of the remaining females,

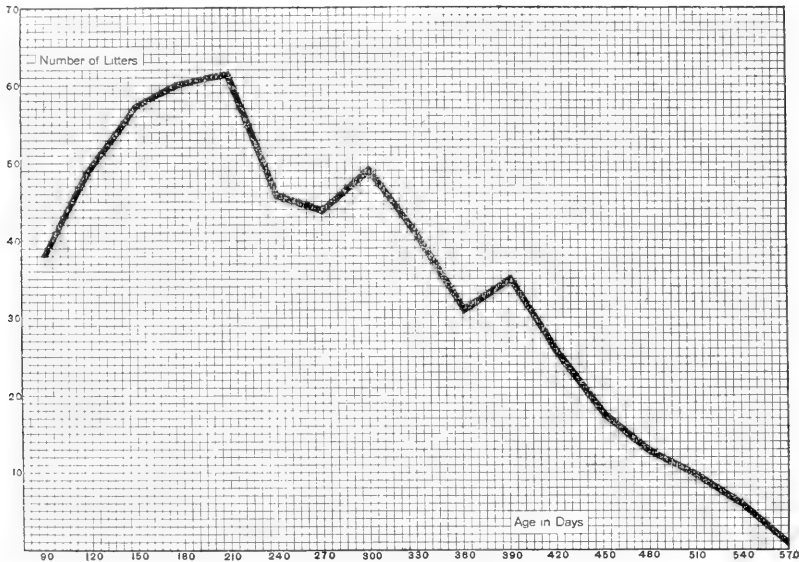


Fig. 1 Graph showing, for the entire series, the relation of the age of the mother to litter production (data in table 2).

with four exceptions, threw litters before they reached the age of 135 days. As table 2 shows, the number of litters cast increased with the age of the mothers until the females attained the mean age of 210 days. After the age of maximum fertility was passed the number of litters cast decreased rapidly, and only a small proportion of the females bore young after they had reached the age of fifteen months.

The graph in figure 1, constructed from the litter data in table 2, shows the relation of the age of the mother to litter production.

The graph in figure 1 starts relatively high and rises rapidly to its maximum which comes at the 210 day period. The decline of the graph is much more gradual than its rise, and not until near the 360 day period does the graph drop to the level at which it starts. From this point the fall is more rapid, and the graph reaches zero after the females have attained the mean age of 570 days. Fecundity in the rat, measured solely by the number of litters cast by the females at different age periods, is thus found to accord remarkably well with the law formulated by Marshall ('10): "The fecundity of the average individual woman may be described, therefore, as forming a wave which, starting from sterility, rises somewhat rapidly to its highest point, and then gradually falls again to sterility." There can be no doubt that animals, in general, tend to follow a similar law, as the litter records for various species collected by Marshall, by Pearl ('13) and others have already shown.

Judging from the data in table 2 a female rat reaches the height of her reproductive capacity when she is about seven months of age. This age represents also the median point in the animal's breeding career. That is, one-half of the total number of her offspring are produced by the time she has reached this age and one-half are produced afterwards.

When the females have reached the age of eighteen months their reproductive activity is usually at an end, as the data in table 2 indicate. Donaldson ('06) has shown that the first year of a rat's life is approximately equal to thirty years of human life. On this assumption a female rat that is eighteen months of age corresponds physiologically to a woman of forty-five. The menopause evidently takes place in these two forms at about the same period in the life span of the individual, but there is no corresponding likeness as regards the age of puberty or of maximum fertility; both of these processes take place in the rat at a relatively much earlier period.

The third column of table 2 shows the average size of the litters cast by the females at different age periods. The litters of very young females contained an average of 6.9 young per litter. This is a smaller average number of young than is found

for any group of litters until the females have past the zenith of their reproductive activity. Such a result was to be expected, since a number of investigations, for instance those of Minot ('91) on guinea pigs and of Hammond ('14) on rabbits and pigs, have shown that the number of offspring produced by young animals breeding for the first time is usually below the number that is considered normal for the species, and also that litter size tends to increase for a time with the age of the female. The largest litters in the series were those produced by females with a mean age of 120 days. Litter size remained close to the maximum until the females were eight months old when a slight diminution in the number of offspring was noticed. A further decrease to an average of only six young per litter was found in the litters thrown by females that were one year old. Each succeeding month added to the female's life seemed to lessen the number of her offspring to a marked extent, and after the females were fifteen months old the mean size of the litters cast was only about three young per litter. Not infrequently the offspring of old females were born dead or soon died from neglect as the mothers seemed unable to suckle them.

There is, as yet, no standard for litter size in 'extracted' strains of rats with which the present series of records can be compared. Miller ('11) and Crampe ('84) give 10.5 as the average number of young in a litter of wild gray rats; but Lantz ('10), on examining a large series of animals, found an average of only 8.1 embryos in pregnant gray females. According to Crampe the average litter of albino rats contains 6.3 young; data for over 1000 litters, collected by King and Stotsenburg, give the mean number of young in albino litters as 7.0. According to the above observations litters of gray rats contain a greater average number of young than do those of albino rats. The 585 litters used in the present investigation contained an average of only 6.7 young. This seems to indicate that litter size in 'extracted' strains of rats is less than that in either of the pure strains from which the animals were derived. It must not be forgotten, however, that the litter size for the pure strains, as given above, was not obtained from the complete breeding rec-

ords of a number of females but from a random collection of litters cast by females of unknown age. Litter size in various strains of rats cannot be properly compared until litter records for the several strains have been collected in a similar manner.

The relation between the age of the mother and litter size is shown by the graph in figure 2. The data used in constructing this graph are given in table 2.

The graph reaches its maximum when the females are practically at the beginning of their reproductive activity (i.e., at

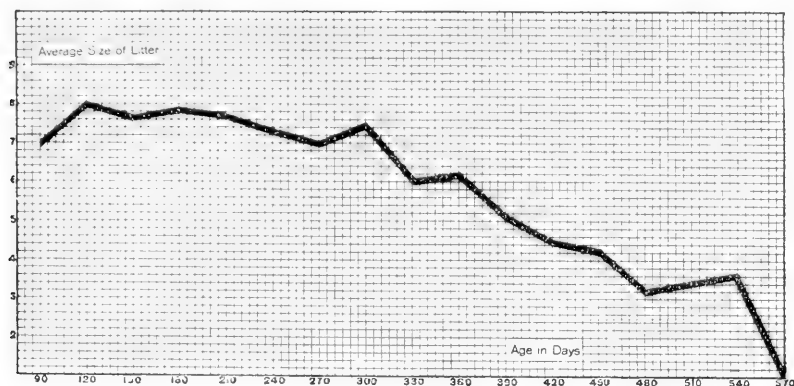


Fig. 2 Graph showing, for the entire series, the relation of the age of the mother to the average size of the litter (data in table 2).

120 days of age), and then declines very gradually approximating zero when the females are eighteen months old. Fertility in the rat, measured by the size of the litters cast, is thus found to be correlated with the age of the mother at the time that parturition occurs.

There is a possibility that the number of the pregnancy is a factor that influences the size of the litters cast. In order to analyze the data on this basis the records have been arranged according to the position of each litter in a litter series and are given in table 3.

When the data are arranged as in table 3 it is found that the second litter is the largest of the series. This result is in accord with the observations of Crampe ('84) and of King and Stotsen-

TABLE 3

Showing the number, the average size of the litters and the sex ratios when the data are arranged according to the position of each litter in a litter series

POSITION OF THE LITTER IN A LITTER SERIES	TOTAL NUM- BER OF LITTERS	AVERAGE NUMBER OF YOUNG PER LITTER	TOTAL NUM- BER OF IN- DIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
1	76	7.2	553	290	263	110.2
2	76	7.7	591	292	299	97.6
3	76	6.9	531	276	255	108.2
4	74	7.3	543	270	273	98.9
5	66	7.0	463	235	228	103.0
6	56	6.8	384	197	187	105.3
7	43	6.9	300	157	143	109.7
8	36	5.8	210	118	92	128.2
9	30	5.0	151	89	62	143.5
10	21	4.8	100	57	43	132.5
11	14	4.8	67	31	36	86.1
12	9	3.8	35	15	20	75.0
13	7	3.5	25	9	16	56.2
14	1	2.0	2	0	2	
	585	6.7	3,955	2,036	1,919	106.1

burg ('15) on the albino rat. The number of the pregnancy, up to five, does not seem to have a very marked effect on litter size. The first five groups of litters have an average of 7.2 young per litter, which is above the average size of litters of albino rats (7.0 young per litter) and considerably greater than the mean size of all the litters in the present series (6.7 young per litter). A slight decrease in size is noted in the sixth litter group, and in the succeeding litters the number of young diminishes steadily. Only exceptionally vigorous females are able to produce more than ten litters and these later litters rarely contain more than one to three young.

As a rule female rats begin breeding when they are three months old, and they will produce a litter each month for several succeeding months if they are in good physical condition. The second litter is cast, therefore, when the female is about four months old and the fifth litter is born when the mother is seven or eight months old. On referring to table 2 it is found that litters born when the females are four months old have a

greater average size than litters cast at any other age period, and that females reach the climax of their reproductive activity at about seven months of age. In both tables there is a rapid decrease in the size of the litters towards the end of the series. As far as the question of litter size is concerned the two tables are in complete agreement. Such a litter series as that in table 3 is necessarily an age series, and it is very probable that it is the age of the female and not the number of the pregnancy that is a determining factor for litter size.

The size of a newborn litter of rats depends, primarily, on the number of ova shed at a given period of ovulation that are capable of fertilization. Litter size, however, is not always indicative of the actual fecundity of the female, since the offspring born represent only that portion of the fertilized ova that were capable of normal development. Not infrequently the examination of a gravid female will show one or several fertilized ova in the uterus that are more or less atrophic and so incapable of developing into normal embryos (Huber, '15). Such ova are usually absorbed *in situ*, and only very rarely are monstrosities found among the normal newborn young. According to Hammond ('14), the lower fertility of young sows as compared with that of adult animals is due to the fact that not so many ova are shed at each period of ovulation. This explanation for the change in the fertility of swine is doubtless applicable also to a similar change in the fertility of rats and of other animals. Very probably the lessened fertility of old animals as compared with that of animals in their prime is due to the same cause. Whether abnormal ova are more frequent in old females than in young ones and so help to diminish the fertility in later life has not, as yet, been determined.

The last column of table 2 gives the coefficients of variation for the size of the litters cast by the females at different age periods. These coefficients show that size variation is considerably greater in the litters thrown by very young females than in the litters produced by females at the height of their reproductive activity when they are seven months of age. The latter

group of litters has the lowest coefficient (25.2) in the entire series.

As the number of litters cast after the females were a year old was relatively small, the coefficients for later litter groups can have little value. There seems, however, to be a very marked tendency for litters cast by older females to exhibit a greater range of variability in size than is shown by the litters of young females, the maximum variability appearing in the litters produced by females when they were about sixteen months old.

The entire series of litters gives 38.00 as the coefficient of variation for litter size. This coefficient is practically the same as that for litter size in the mouse, which is 37.5 according to the records collected by Weldon ('07), but it is 10 points less than the coefficient for the number of human offspring (Powys, '05). The coefficient of variation for fertility is very high in all mammals, apparently, being at least 25 per cent in the several cases where it has already been determined (Surface, '08).

Different females—even sisters from the same litter—show marked variations in the number and in the size of the litters they produce. Whether such differences depend upon the inheritance of various fertility factors, or whether they are due to environment or to individual peculiarities of the females themselves remains to be determined.

Table 4 shows the number of litters produced by the seventy-six females whose breeding records are used in the present study.

As shown in table 4, the range of variation in the number of litters produced by different females was from three to fourteen with an average of 7.7 litters per female. One of the two females that cast only three litters did not breed until she was six months old when she gave birth to a litter of seven. A second litter, with nine young, was born when the mother was eight months old, and a final litter, containing seven young, one month later. This female lived to be seventeen months old and she appeared to be in good physical condition until shortly before her death. The other female casting only three litters had a very similar breeding history. Some diseased

TABLE 4
Showing the litter production of 76 female rats

NUMBER OF BREEDING FEMALES	NUMBER OF LITTERS CAST
2	3
8	4
10	5
13	6
7	7
6	8
8	9
9	10
4	11
2	12
6	13
1	14
76	585

condition of the generative organs was doubtless responsible for the small number of litters produced by these two females, as investigations being carried on in the animal colony of The Wistar Institute by Dr. Stotsenburg show that sterility in a female rat is usually due to the formation of ovarian cysts or to degenerative changes in the uterus.

According to Crampe ('84), female albino rats, as a rule, do not produce more than four or five litters: records collected by Miller show that the wild gray rat has relatively more litters than the albino rat. The average of 7.7 litters per female, found in the present series of animals, is undoubtedly too high for the general run of females. Twenty-three of the seventy-six breeding females in this series had a total of five or six litters only, and it seems probable that this is about the average number of litters produced by female rats in general.

While six females had thirteen litters each, only one female gave birth to fourteen litters. This latter case is so unusual that it seems worthy of special note. The complete litter data are given in table 5.

This female, a piebald, gave birth to her first litter on February 7 when she was ninety-five days old. This litter was ex-

TABLE 5

Showing the litter production of a female piebald rat, that was born November 4, 1913, and died June 14, 1915

LITTER SERIES	DATE OF BIRTH	NUMBER YOUNG	MALES	FEMALES
1	February 7, 1914.....	11	5	6
2	March 11, 1914.....	13	5	8
3	April 3, 1914.....	8	6	2
4	April 30, 1914.....	9	5	4
5	May 23, 1914.....	9	6	3
6	June 20, 1914.....	10	2	8
7	July 14, 1914.....	11	5	6
8	August 12, 1914.....	6	2	4
9	September 10, 1914.....	10	6	4
10	October 15, 1914.....	10	9	1
11	November 23, 1914.....	4	3	1
12	January 28, 1915.....	3	2	1
13	March 26, 1915.....	3	3	0
14	April 28, 1915.....	2	0	2
		109	59	50

ceptionally large for the first litter of so young a female as it contained eleven young. The second litter, with thirteen young, was cast the following month. It is rather remarkable that both of these litters should be so much larger than normal, since, as a rule, a very large first litter is followed by a comparatively small one, unless at least two months intervene between the birth of the litters. The female cast two litters in April, and subsequently she gave birth to a litter each month until she was twelve months old. With one exception each of these litters was larger than the average litter of albino rats. A marked decline in fertility was noted after the female was a year old: the intervals between litters became longer and the size of the litters decreased. The fourteenth litter, which contained only two young, was cast when the female was about seventeen months old, and although the female lived to be nearly twenty-two months old she did not breed again. During this long period of reproductive activity a total of 109 young were born, 59 males and 50 females. The median point in this female's breeding career was the same as that for the entire group of

females, namely seven months, and she produced an average of 7.8 young in each litter.

An examination of the individual records for each of the remaining females in the series that gave birth to a very large number of litters i.e., from eleven to thirteen, shows that in every instance the first litter cast was large, containing from nine to eleven individuals. In those cases where females produced less than six litters the first litter cast, with one exception, never contained more than seven young. The number of records is so small that no definite conclusions can be drawn from them, but they seem to indicate that the size of the first litter cast is somewhat of an index of the fertility of that particular female: a large first litter indicating that the female, if she keeps in good physical condition, will produce more litters than the average run of females. Crampe states that the second of a rat's litters is always the 'best' and that this litter is indicative of the size of subsequent litters. This observation has been confirmed only in part by the present series of records: the second litter is the largest of the series, but the size of this litter is not as indicative of the later fertility of the female as is the size of the first litter cast.

Individual rats show as marked differences in the number of young produced at one birth as they do in regard to the total number of litters cast. Litters cast by some females are almost always relatively large. The female whose litter record is given in table 5, for example, cast but one litter in the first ten that contained less than seven young. Some females never have a litter that contains more than seven young, while others females cast a large and a small litter alternately.

The litter frequencies in the three series of rats are shown in table 6, the range in litter size being from one to sixteen.

In table 6, as in table 1, there are slight differences in the corresponding data for the three series of rats that may or may not prove to be significant when larger series of records are analyzed. Litters of eight young were most frequent in the piebalds and in the extracted grays, while six was the most common number of young in the litters of extracted albinos. The data for the

litter frequencies in the combined series is shown in the form of a frequency graph in figure 3.

The graph in figure 3 has two modes, one at the point of six and the other at the point of eight young per litter. The graph thus appears to be compound, and it is possible that one of the two modal points corresponds to the degree of fertility normal for the wild Norway rat and the other to the degree of fertility that characterizes the albino rat, since these are the two strains from which the animals used for this study were derived. As the material is probably heterozygous as regards the factors for litter size, it does not seem advisable to attempt any analysis of the curve. It is of interest in this connection to note that the graph for litter frequencies in swine, as given by Went-

TABLE 6
Showing litter frequencies in the three series

SIZE OF LITTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16
Piebalds.....	6	20	32	35	31	56	40	71	48	28	17	12	8	1	1
Extracted albinos.....		5	8	7	9	20	14	8	12	4	1				
Extracted grays.....		3	9	6	13	12	10	15	11	9	1	1	1		
	6	28	49	48	53	88	64	94	71	41	19	13	9	1	1

worth and Aubel ('16), has three modal points; one at four, a second at eight, and a third at twelve pigs per litter. The first mode corresponds to the degree of fertility in the wild hog, the third is close to that of the most fecund of the domestic breeds of swine, and the third probably represents a heterozygous condition.

Evidence regarding the relation of the age of the mother to the sex of her offspring is conflicting. Statistics collected by Bidder ('78) and by Punnett ('03) show that there is a great excess of boys among the children of very young mothers, the relative number of boys decreasing at subsequent births until the mother is thirty. Among children of old mothers (i.e., over forty) the sex ratio is again very high. In the horse Wilchens ('86) found a relation between the age of the dam and the sex of her offspring very similar to that existing, apparently,

in the human race. On the other hand, Schultze's ('03) investigations on mice indicate that the age of the mother has seemingly no influence whatever on the sex of her young.

According to the observations of King and Stotsenburg the normal sex ratio in the albino rat is about 107.5 males to 100 females. As there are no available data regarding the normal sex ratio in other strains of rats the sex ratio in the albino rat is here taken as the standard with which to compare the sex ratios found in the present series of animals.

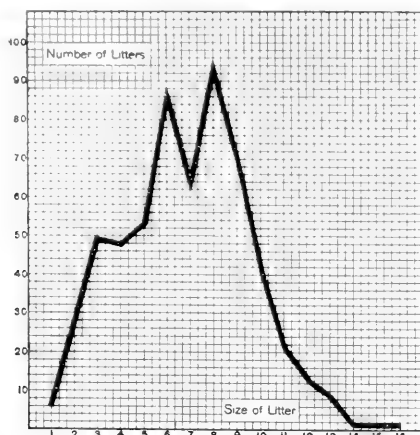


Fig. 3 Graph for the frequencies of litter size in the entire series (data in table 6).

Table 2 gives the sex ratios for the various litter groups when the data are arranged according to the mean age of the females at the time that the litters were cast. The sex ratios in litters belonging to closely related groups are so unlike that it would appear that there is no relation whatever between the age of the mother and the sex of her offspring. The sex ratio for the entire series of 3955 individuals is 106.1 males to 100 females. This shows that in the strains of rats used for this study the normal proportion of the sexes is about the same as that in the pure albino strain.

When the litter data are arranged according to the position of each litter in a litter series (table 3), the sex ratios obtained

for the individuals in successive groups of litters are not quite as diverse as those for related litter groups as shown in table 2. The sex ratio among the individuals belonging to the first litters of the series is higher than the standard, and in subsequent litter groups, up to the fifth, there is seemingly a tendency for the number of male offspring to decrease. A similar change in the sex ratios from the first to the fourth litter was noted by King and Stotsenburg in a series of litters cast by twenty-one albino females. Beginning with the fifth litter the sex ratios rise gradually until a maximum of 143.5 males to 100 females is reached at the ninth litter of the series. For the eleventh and subsequent litters, however, the sex ratios are much lower than the standard. From the sex ratios as given in table 2 it would appear that among the individuals of a litter series the sex ratio might be expected to start relatively high and then fall steadily until about the fifth litter, rise again gradually to a maximum at about the ninth or tenth litter and subsequently drop to a low level which is maintained until the female reaches the menopause.

The records under consideration are a special group selected solely because they cover the complete breeding history of a number of females that lived to an advanced age. Perhaps, therefore, they cannot be used legitimately to give evidence regarding the possible effects of the age of the mother on the sex of her offspring. From the data as given the only conclusion that can be drawn is that the age of the mother is not a dominant factor in determining the sex of her young. If, as Riddle ('16) maintains, sex is determined by the 'level of metabolism' in the fertilized egg, there is a possibility that the age of the mother may indirectly influence sex through its effects on the metabolic processes in the egg. Age has a profound influence on every tissue in the body, and its effects on the germ cells is a problem that must be attacked from a chemical standpoint, since it can never be solved by sex statistics however extensive they may be.

SUMMARY

1. Litter data covering the entire breeding history of seventy-six female rats are given in the present paper. All of the females belonged to 'extracted' strains that were derived from the F_2 generation of a cross between the wild Norway rat and the domesticated albino.

2. The material used comprises the data for 585 litters containing 3955 individuals, 2036 males and 1919 females. The average number of young in each litter was 6.7.

3. Fertility in the rat, measured by the total number of litters cast, increases with the age of the female up to the time that the animal is seven months old. There is a sharp decline in fertility after the female is a year old and, except in rare instances, the menopause has appeared by the time that the female is eighteen months of age.

4. Female rats reach the height of their reproductive activity when they are about seven months of age. This age also represents the median point in the animal's breeding career.

5. The age of the mother is a factor in determining the size of the litter cast. Litters of very young mothers are relatively small, and later litters are large until the female reaches seven months of age. Litter size diminishes with the reduction in the number of litters cast, and litters of very old females rarely contain more than three young.

6. The second litter is the largest of the series, the third and fourth litters are usually a little larger than the first.

7. The serial number of the pregnancy, up to the fifth, does not seem to alter the size of the litter to any great extent. The sixth litter cast, however, is smaller than the preceding ones, and the number of offspring decreases rapidly as the position of the litter in the litter series advances. It is very probable that it is the age of the mother, not the number of the pregnancy, that influences the size of the litters.

8. Coefficients of variation for litter size show that the litters cast by very young females have a greater range of variation in size than have the litters cast by females at the height of

their reproductive activity. From this point the range of variation in litter size appears to increase as the female grows older, and to reach its maximum in the litters cast when the females are sixteen months old.

9. For the entire series of litters the coefficient of variation for litter size is 38.00.

10. The total number of litters produced by different females varied from three to sixteen, with an average of 7.7 litters per female.

11. The majority of female rats probably produce from five to six litters only.

12. The size of the first litter cast seems to be somewhat of an index of the fertility of the female. If the first litter is very large the female will probably cast more litters than the average run of females, provided she remains in good physical condition.

13. The range in litter size was from one to sixteen. Eight was the most frequent number of young in the litters of the piebalds and of the extracted grays, while six was the most common number for the litters of the extracted albinos.

14. The sex ratio for the 3955 individuals in the series was 106.1 males to 100 females. This sex ratio is very close to the normal sex ratio for the pure albino strain (107.5 males to 100 females).

15. The sex ratios obtained for the various litter groups (tables 1 and 2) do not indicate that the age of the mother is a dominant factor in determining the sex of her offspring. Old females, however, seem to produce relatively more females than male young.

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TECHNIQUE NOTES

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I. THE APPLICATION OF BENDA'S NEUROGLIA STAIN

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This note embodies the results of some experiments with Benda's stain for neuroglia cells and fibers. The stain is, as is well known, the basic anilin dye, toluidin blue, used after a double mordantage of the sections in ferric alum and sodium sulphalazarinate. A previous mordantage of the tissues in a chrome solution, a 'chromation,' is an essential part of the method. Benda himself has employed two methods for producing this 'chromation.' The first (Benda, '00) was the use of Weigert's chrome alum bath followed by chromic acid, with tissue fixed in formalin. In the second method (Benda, '10), tissue fixed in alcohol was placed in 10 per cent nitric acid, then in 2 per cent potassium dichromate, and finally in 1 per cent chromic acid.

My results show that this 'chromation' may be obtained by using a chrome fixer, or better, by a dichromate bath after fixation; and the best results are produced by the use of a fixer containing a dichromate, followed by a dichromate mordantage. This is essentially the method used in preparing tissues for Weigert's copper hematoxylin, and it may be said that in general, sections which will take a good Weigert stain for myelin will also give good results with Benda's neuroglia stain. Tissues fixed in Zenker's fluid, in Zenker's followed by Müller's, in Helly's (Zenker-formol) followed by Müller's, in Müller's alone, in formalin followed by copper dichromate, and in the copper dichromate-sublimate-acetic mixture as used by Kingsbury, followed by copper dichromate, all have given good preparations with Benda's stain. It is worthy of note that the tissue fixed in Zenker's fluid alone gave the poorest neuroglia stain, and that good Weigert preparations were obtained from all the tissues, except this same one.

A number of sections of the spinal cord of an animal (a skunk happened to be available at the time) were fixed in copper dichromate-sublimate-acetic and mordanted in copper dichromate for periods from two to twenty-five days to determine the length of time required for the best 'chromation.' These all gave good results with Benda's stain but the best preparations were obtained from tissues mordanted four to six days. Sections of this same material were also stained with Weigert's copper hematoxylin, and good preparations were obtained

from the tissue mordanted two days. After a longer stay in the copper dichromate solution, the myelin failed to stain with the copper hematoxylin, remaining yellow, while the neuroglia cells and fibers stained blue. With Benda's stain, the myelin after a short mordantage stains a light reddish brown, but after a longer mordantage remains yellow, unaffected by the stain.

From this it would seem that a short mordanting in dichromate solution is best for staining the myelin and a longer is best for neuroglia. After a prolonged mordantage the axis cylinders are brought out more clearly, apparently as a result of their 'chromation' for they did not stain with the copper hematoxylin. This does not quite agree with the results of Smith, Mair, and Thorpe ('08) who state that the order is myelin, axis cylinders, neuroglia.

This method of fixation—Helly's fluid (Zenker-formol) followed by Müller's or copper dichromate-sublimate-acetic followed by copper dichromate—has given excellent results with tissues from a number of animals: cat, dog, man, mouse, rat, skunk. The spinal cord was used in each case, and in addition, in one form (dog) the optic nerve was taken.

These results would show that for the 'chromation' four to six days in copper dichromate (2.5 per cent solution) is equivalent to two to four weeks in Müller's fluid or plain potassium dichromate solution. There is apparently little choice between them; possibly the tissues mordanted in copper dichromate are a trifle less brittle. A 2 per cent solution of potassium dichromate works as well as Müller's fluid.

My results with material fixed in this way (Müller's after Helly's or copper dichromate after the copper dichromate-sublimate-acetic mixture) have been better than with tissues treated as Benda recommends. The preparations correspond to his description; neuroglia fibers and nuclei of neuroglia cells, deep blue; cytoplasm of neuroglia cells, paler blue or purplish; myelin, reddish brown; axis cylinders darker red; connective tissues light red or pink; nerve cells reddish or purplish; and Nissl bodies darker purple.

Benda's method of staining was used, and is given for reference. Paraffin sections 5 to 8 μ are treated as follows:

1. 4 per cent ferric alum, twenty-four hours.
2. Running water, ten–twenty minutes, followed by several changes of distilled water.
3. Amber-yellow solution of sodium sulphalizarinate, twenty-four hours (saturated solution of sodium sulphalizarinate (Kahlbaum) in 70 per cent alcohol, 1 cc., distilled water, 100 cc.).
4. Several changes of distilled water, which is then absorbed with tissue paper.
5. 0.1 per cent aqueous solution of toluidin blue, heated (on the slide) until it steams, and allowed to cool, fifteen minutes or more.
6. After rinsing in distilled water the sections are treated for a few seconds with acidulated alcohol (70 per cent alcohol, 100 cc., concentrated hydrochloric acid, 6 drops). The length of time required varies

with the different methods of mordanting, and is best determined by trial.

7. After the acid alcohol is absorbed with tissue paper, the sections are rapidly dehydrated with absolute alcohol.

8. The sections are then differentiated with creosote under control of the microscope. This usually takes several minutes. If more than ten seems necessary, it may be well to remove the creosote with absolute alcohol and treat with acid alcohol again for a few seconds; then the sections may be dehydrated and differentiated with creosote as before.

9. The creosote is absorbed with tissue paper and after several changes of toluene or xylene, mounted in balsam or damar. To prevent the fading of the stain, it is necessary to remove the creosote pretty thoroughly.

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II. SOME USES OF MALLORY'S CONNECTIVE TISSUE STAIN

H. M. KINGERY

Mallory's anilin blue connective tissue stain is of course well known for the purpose for which it was intended, but it has been found quite useful for other purposes as well. As usually employed for collagen fibers, Zenker material is used. I have found that this stain used with tissue fixed in picro-aceto-formal (Bouin's fluid) gives a very pretty differentiation for skeletal muscle, distinguishing clearly the isotropic and anisotropic bands.

In practice, a thin muscle is moderately stretched and pinned out flat on a cork which is then floated upside down on the fixer. The picric acid is sufficiently removed after washing about a week in alcohol. Thin paraffin sections (4-6 μ) are stained according to Mallory's directions (five minutes in the acid fuchsin solution and then twenty minutes in the anilin blue solution; differentiation in 95 per cent alcohol, dehydration in absolute alcohol, toluene or xylene, balsam or damar). Differentiation proceeds rather slowly and may be watched under the microscope. In a finished preparation the dark band (anisotropic) is stained blue and the light band (isotropic) is light red or pink. Hensen's disc (M) appears light in the middle of the blue band and Krause's membrane (Z) is deep red in the middle of the pink band.

This method of fixation and staining when applied to insect material differentiates very nicely chitinised from non-chitinised cuticula. The chitinised is stained red and the non-chitinised is a clear blue. This method was applied particularly to the intestine of the grasshopper and besides differentiating the cuticula it brought out the striations of the muscle fibers of the muscular coats with almost diagrammatic clearness.

With this same fixation (picro-aceto-formol) Mallory's stain also brings out the connective tissue very clearly. And I have found that the stain may be used with good results after a number of fixers—alcohol, Carnoy's 6-3-1, formalin—if the sections are placed for a short time in picro-aceto-formol and then washed, before staining. Aqueous and alcoholic solutions of picric acid also give fairly good results as 'mordants,' but not so good as picro-aceto-formol. The results obtained by this method compare very favorably with those secured after Zenker fixation.

The formulae follow.

Picro-aceto-formol

Saturated aqueous solution of picric acid	75 parts
Formalin.....	25 parts
Glacial acetic acid.....	4 parts

Mallory's anilin blue stain

Solution A.	
Acid fuchsin.....	0.2 gram
Distilled water.....	100.0 cc.
Solution B.	
Grübler's water soluble anilin blue.....	0.5 gram
Orange G.....	2.0 gram
1 per cent aqueous solution of phosphomolybdic acid.....	100.0 cc.

III. THE USE OF THE VAN WIJHE METHOD FOR THE STAINING OF THE CARTILAGINOUS SKELETON

GUSTAVE J. NOBACK

While the Schultze and the Spalteholtz clearing methods for bone are well known and frequently used, it would seem to the writer that the van Wijhe staining and clearing method for cartilage, which nicely complements the Spalteholtz method in the study and demonstration of the development of the skeleton, is not so well known and appreciated. I venture therefore to call attention briefly to its value, having used it during the past three years with satisfactory results.

The method is very simple; embryos or other material to which it is to be applied should be preserved in alcohol or (better) formalin. The specimen is next placed in 67 per cent alcohol with 1 per cent of hydrochloric acid added, for several days or a week. It is then transferred to the same solution plus 0.25 per cent of methylene blue in which stain it remains for a week or two weeks, until thoroughly stained. Toluidin blue may be used instead of methylene blue if preferred (Lundvall

'04,'12). It is then retransferred to the acid alcohol, which is changed at intervals of one or two days or when markedly colored. In this the specimen remains until the color is nearly entirely removed from all parts save the cartilage which remains deep blue. To remove the acid it should be washed for several days with changes of 82 per cent (85 per cent) alcohol and then dehydrated by passing up through 95 per cent alcohol, absolute alcohol, equal parts of absolute alcohol and benzene, into benzene, changed at least once, in which it may remain, or it may be mounted in xylene damar or Canada balsam.

The method is particularly serviceable in demonstrating the development of (a) the sternum and ribs, (b) the auditory ossicles, Meckel's cartilage and Reichert's cartilage, (c) the chondrocranium, (d) the cartilage in the developing bones of the extremities, etc.

As has been indicated at the beginning, the method supplements satisfactorily the Spalteholtz method in which the bone has been stained red in the usual way by means of alizarin. Two embryos, or the two halves of the same embryo carefully cut as nearly as possible in the median plane, may be run through, for bone (according to the Spalteholtz alizarin method) and for cartilage respectively, the skin, central nervous system and viscera having been removed to clarify the view. One arm or leg may be stained for bone and the other for cartilage, etc.

A solid mounting medium offers so many advantages over a liquid one that where possible it was used, damar balsam in xylene solution being preferred to Canada balsam because of its lighter color. By using glass supports for the cover-glass, such as small pieces of glass rod, etc., and using care in adding successive amounts of xylene damar, solid mounts of quite large specimens may be made on glass slides or plates. In this way specimens such as arms of the same embryo stained for bone and cartilage respectively may be mounted side by side or in parallel series to show advancing stages of development. Glycerin-jelly may be used as a solid mounting medium in the Schultze method, or with alizarin stained bone, but it may not be used with specimens stained for cartilage with methylene or toluidin blue. In the use of liquid mounting media, it was found that the benzyl benzoate and oil of wintergreen mixtures used in the Spalteholtz method were not so useful for mounting specimens stained for cartilage by the van Wijhe method, since fading was apt to result. Benzene was found to be the most serviceable mounting medium. Lundvall has used for larger specimens benzene four parts and carbon disulphide one part (see also Lundvall, 1912).

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IV. A CONVENIENT METHOD OF ORIENTATION IN PARAFFIN IMBEDDING WHEN PAPER TRAYS OR BOXES ARE USED

B. F. KINGSBURY

The following simple method has been used by the writer occasionally for the past fifteen years or so and since I do not recall ever having seen it mentioned, a brief description may not be out of place. The paper box method of paraffin imbedding which I use quite generally is of course well known and described in most books of technique (cf. Lee, *Vade-mecum*, p. 77).

The procedure in obtaining the orientation is the following. By means of a moderately soft lead pencil direction lines or, if desired, the outline of the specimen or embryo (traced from a $\times 1$ photograph), are marked on the inside of the bottom of the box chosen or usually on the paper before it is folded. The box is then floated on cold water and the melted imbedding paraffin poured in. The specimen is then at once transferred from the melted infiltration paraffin to the box. By the time this has been done a thin translucent layer of solidified paraffin covers the bottom of the box, the orientation lines upon the paper showing through. The specimen is arranged as desired according to the orientation lines or upon the outline and the paraffin allowed to cool, etc. Subsequently after removing the paper from the cold block it will be found that the pencil mark is on the paraffin block which then may be trimmed and by it easily oriented for sectioning.

ON THE ELECTIVE STAINING OF THE ERYTHROCYTE

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Today we are in possession of numerous excellent staining methods applicable to microscopic researches of the blood. The anilin dyes have been especially serviceable in the coloration of the red blood cells, and among these Eosin and Orange G deserve special mention. Solutions combining a number of dyes, for instance the triacid mixture (Ehrlich-Biondi), eosin methylblue (May-Grünwald), methylazure methylblue eosin (Giemsa), have rendered excellent service in the hands of many investigators.

However these dyes are designed more particularly for the staining of film specimens of blood, since in staining sections they color not only the erythrocytes, but also the plasmic substances; in a strict sense they are, therefore, not the elective stains for erythrocytes. The need of an elective stain for the hemoglobin bearing erythrocytes is often felt in researches dealing with the genesis of blood cells and in many other types of the histological investigations.

I have recently discovered a method of the elective tinction of the erythrocyte. The finding is based on the fact that the phosphomolybdic acid lac of alizarin stains among several tissue elements only haemoglobin. After mordanting with phosphomolybdic acid the great majority of the tissues of the animal body lose the property of staining with molybdenum alizarin lac while the erythrocyte or more particularly haemoglobin is colored with it. In this regard the method here recorded may be regarded as a new method for the microscopic determination of haemoglobin.

The various steps of the method are as follows:

The material may be fixed in formalin, sublimate, potassium bichromate, etc.

1. The sections are transferred to distilled water.

2. Mordant in 10 per cent phosphomolybdic acid solution for 30 seconds to 2 minutes.

3. Wash in water.

4. Stain in following mixture for 20 minutes to 20 hours. Sodium sulfalizarinate, saturated aqueous solution—100 cc. 10 per cent phosphomolybdic acid, aqueous solution—30 cc. (10–50 cc.).

5. Wash in water.

6. Alcohol.

7. Xylol, balsam.

It is not necessary to prepare the staining solution a short time before using. A solution kept for one-half year, exposed to daylight, gave excellent results. On mixing the phosphomolybdic acid and sodium sulfalizarinate solutions the yellowish brown color first observed later changes to one of bright orange red.

Attention is called to the fact that on staining sections according to this method, the erythrocytes of vertebrates, the nuclei of the erythrocytes excepted, are durably stained a light to dark orange red, other tissues remaining unstained. The method is thus differential. Some-

times the nuclei and protoplasmic substances, especially in materials fixed in bichromate of potassium, take the stain a little, but it is easy to distinguish the bright orange red color of erythrocyte from the dirty yellowish brown color of nuclear chromatin or from other protoplasmic substances, for in the latter the color bleaches gradually. The connective tissue fibrils and osseous tissue are colored a bluish tinge, increased in intensity by longer staining so that excellent double staining, with brilliant contrast in orange red and blue may be obtained.

It remains to be considered whether the molybdenum alizarin lac stains haemoglobin or some other structure of the erythrocyte. To determine this question the following experiment was undertaken.

On a spot on two slides the chemical pure hemoglobin (Merk, Darmstadt), was spread and near it a section of liver fixed in formalin. Both were allowed to dry. One slide was now mordanted in the phosphomolybdic acid solution, the other not. Both were then stained in the alizarin molybdenum lac. On the slide mordanted we observe that the liver cells were not stained while the haemoglobin was colored a very dark orange red. On the unmordanted slide the liver cells were colored brilliant red and the haemoglobin a deep orange red. This observation may serve to show that the erythrocyte or haemoglobin represent the substance which stains after mordanting of phosphomolybdic acid by molybdenum alizarin lac.

From the facts given it would seem that molybdenum possesses the property of effecting animal tissues so that mordanting by it diminishes or entirely deprives them of their staining property, this with the exception of haemoglobin. It is a question whether the molybdenum alters the majority of tissues, haemoglobin excepted, or whether there exists a peculiar affinity between the haemoglobin and this lac. It may be of interest to determine precisely the chemical relations of both.

It is recommended that before or after the staining by the solution given the sections be treated with some nuclear dye. Haematoxylin may be used for this purpose, the section being first stained in this dye. On the subsequent use of haematoxylin, staining of connective tissue fibrils is obtained by reason of the formation of an haematoxylin molybdenum lac as in the Mallory's stain.

In conclusion it may be stated that as with the solutions so with the stained preparations, they are durable. The slides made one year ago and kept in the half dark are as yet unbleached.

After the present work was completed and ready for publication, attempts to make alcoholic solutions were undertaken. The alcoholic saturated solution of the sodium sulfalizarinate changes a little its color on the addition of the phosphomolybdic acid. The procedure is the same as described for aqueous solutions. By this modification the length of time required for staining has been considerably shortened and the coloration seems more certain. On the durability of both solutions and preparations, when alcoholic solutions are used, a future communication will give information.

The alcoholic stain has been prepared by mixing the two following solutions: Sodium sulfalizarinate saturated alcoholic solution, 100 cc.; 10 per cent phosphomolybdic acid aqueous solution, 1 to 2 cc.

NEUTRAL RED AS A CELL STAIN FOR THE CENTRAL NERVOUS SYSTEM

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The method described below has been used for eight years for the study of the tigroid content of cells as well as the size, form and grouping of cell bodies. As a 'Nissl method' it gives better results with formalin material than methylene blue or toluidin blue. The method is simple and the stain is permanent and is suitable for photography.

The stain is made up in a 1 per cent aqueous solution and kept for months or years until thoroughly ripened. The ripening process is hastened by exposure to the air or by boiling but I know of no way to obtain a satisfactory staining solution in a few days. A good stain can be had after a few months; a better one after three or four years. A 1 per cent solution is diluted as required and the dilute stain may be used over and over again.

The stain is used with either celloidin or paraffin sections. Differentiation is carried out in alcohol. The lower grades of alcohol differentiate slowly enough to allow one to examine the sections under the microscope. The higher grades wash out the stain and the sections should remain in these grades only as long as necessary to secure dehydration. If differentiation has been completed in 70 per cent alcohol, it may be carried too far while the sections are being dehydrated in the higher grades.

The clearing of the sections requires care, as any alcohol left in the sections will remove the stain. Also, some of the common clearing agents injure the stain while others soften celloidin. For paraffin sections xylol is safe and satisfactory. For celloidin sections castor oil and bergamot oil are the only common clearing agents which have proved constantly reliable. Carbol-xylol ruins the stain at once and cedar oil, clove oil, cajapat oil, oil of thyme, and anilin oil have all been unsatisfactory for one reason or another, at least in certain samples. After clearing in bergamot or castor oil it is well to rinse the sections in xylol. The mounts harden more rapidly and seem a little more brilliant and transparent. If castor oil is used the sections can be handled much more easily if the oil is thinned by addition of one part of xylol to two or three of castor oil. The xylol also counteracts any tendency of the castor oil to soften the celloidin. It is most convenient in handling large sections to float them on glass slides in the last grade of alcohol and pass them through the clearing agent on the

slide. In all these particulars the method is more difficult with large and, especially, thick sections. Sections 50 microns in thickness through the whole brain of a newborn babe have been perfectly stained by this method, while sections 100 microns thick through the entire adult human brain could not be differentiated and cleared uniformly.

The steps in the process may be stated as follows:

Material fixed in formalin or alcohol. Old formalin material gives good results.

Paraffin or celloidin sections.

Aqueous neutral red Grübler (well ripened) diluted to one-fourth or one-tenth of 1 per cent.

Use warm 10 to 30 minutes, or cold 12 to 24 hours. Differentiate in 50 or 70 per cent alcohol. Dehydrate rapidly in higher grades.

Clear paraffin sections in xylol; celloidin sections in castor-xylol or bergamot oil.

Mount in dammar or balsam. Clearing and mounting media must be neutral.

Neutral red does not work well after chromic salts and hence can not be used as a secondary stain after Weigert. The stain does work admirably, however, after either the Cajal or Bielschowsky process on formalin or alcohol material. Some beautiful preparations have been obtained by treating with neutral red as above sections in which the silver stain has attacked chiefly the fibers. This enables one to study the relations of cell masses to fiber bundles and to see the origin of a silver-coated axone from the cell-body stained by neutral red.

BIOLOGICAL PROBLEMS AND THE AMERICAN ASSOCIATION OF ANATOMISTS

HENRY H. DONALDSON

*Address of the President at the meeting of the American Association of Anatomists,
December 27, 1916*

The purpose of this address is to discuss the relations of anatomy—as represented by our Association—to the problems of biology, and to consider our future course in the light of our past record.

My opportunity to deal with these matters has come as a result of your kindness in placing me in the presidential chair. I wish to express my appreciation of this honor which you have conferred, and also to venture the hope that this address may serve to indicate my interest in our common development.

Let me begin with a few words on the historic relations of anatomy.

It is easy to see that the divisions of biology have been named in a rather incongruous way. For example: Zoology is defined by its material; animals. Physiology, by a great domain; nature. Pathology, by a state; disease, and Anatomy, by a mode of procedure; dissection.

Strictly speaking therefore the anatomist is one who cuts up things—with the common connotation that his dissection is applied to the adult human body. But, as these instances show, derivations are hardly illuminating.

As we know, all workers in science are arbitrarily labelled, for as they enter the hall of science they find at the very threshold a robing room. Here hang the gowns mostly of ancient cut; bearing still more ancient labels, and clothed in one of these they pass, each to his appointed place.

It is an arrangement of convenience mainly, but the dead hand of these labels often lies heavy on us and may become even a misdirecting influence.

When we turn to human anatomy as a practice, we find that it began without technological affiliations—as a pure science so to speak—and only later became fundamental to surgery. When that branch of medicine developed, and—despite the absence of anesthetics or the control of sepsis—was pushing forward, with speed in operation as the great desideratum, the cultivation of gross human anatomy as a body of ever ready knowledge was most intense.

With the dawn of the modern era in surgery, its importance as a mass of minute information carried with much effort, diminished, for the facts came to be excellently recorded; the surgeon and the professional anatomists worked together and speed ceased to have its former significance. Moreover anatomy had reacquired something of its earlier and broader point of view.

From the nature of the case human anatomy is purely descriptive, and for various reasons has remained almost exclusively a medical cult. Let us see then how our Association of Anatomists stands in relation to this subject, when it is narrowly defined.

Out of 323 members we have 146, or 45 per cent, connected with departments of human anatomy in the strict sense.

Going a step further and examining the first fifteen volumes of *The American Journal of Anatomy*—where the larger portion of the purely anatomical papers is printed—we find that there have appeared 300 papers in all. These I have analyzed by groups into those dealing with gross human anatomy; with mammalian embryology; and with other topics.

Subdividing this last group into those purely descriptive and those including experimental work.

By this treatment it appears that 11 per cent are on gross human anatomy; 40 per cent on mammalian embryology and 49 per cent on other topics. Thirty-five per cent within the last group being purely descriptive, 14 per cent including experimental work.

In this connection it should be remembered that while *The American Journal of Anatomy* contains almost all of the papers on human anatomy which are presented to us yet the papers on other topics tend to appear in different journals.

That the foregoing proportions represent fairly well what has been going on is indicated by the fact that an analysis of our programs for the same period shows a similar distribution of the papers. In all, there have been 558 titles presented at our meetings and the proportion for human anatomy rises, by virtue of the presentation of several papers on the brain and on anthropometry, to 16 per cent.

Taking then these two determinations for the work on human anatomy at their face value it appears that this subject is represented in *The American Journal of Anatomy* by 11 per cent of the papers, and at the meetings of the Association by 16 per cent of the titles—there being 45 per cent, or nearly half, of the members of the Association who are also members of departments of human anatomy.

The reason for this seeming disparity is not far to seek, for even a hundred years ago we find men like old John Barclay of Edinburgh rather desperate over the anatomical situation.

Before going further however, let me add here a few words intended to forestall any misinterpretation which might be made of the preceding paragraphs.

These paragraphs are not intended to be in dispraise of human anatomy, nor as questioning either its paramount importance, nor the debt we all owe to those who have put the facts in order and made them accessible. Neither by my statistics would I imply that the anatomical members of this Association have failed in loyalty to their subject.

The implications of the old name do hamper us however, and I wished to point that out, by showing, as I have done, how one could apparently demonstrate that the Anatomical Association was not attending to anatomy. On the other hand it was important to emphasize the accepted fact that human anatomy, in the historic sense, has been so carefully worked over and is so strictly descriptive, that today the human

body, though very completely known, is not the material with which we can work most advantageously in the solution of many biological problems. Appreciation of this fact has led to action and there is therefore a tendency to overstep the earlier and narrower bounds, to escape the ancient barriers and to annex the surrounding country. This tendency appears in our programs; but of these matters—later.

The fairest conclusion from such a survey, is this: To any group of active biological workers it is hardly possible to give a descriptive name—fixed by statute, so to speak—which shall hold good for any length of time and be an indication of their intellectual aims for the guiding interests of such a group are bound to change from decade to decade, if not more rapidly.

This Association illustrates most clearly such a shift of interest. Those who founded it (1888), and those who watched over its earlier development, felt constrained to retain the term anatomy—but at the same time sought to give to this term a wide and elastic meaning—a meaning, if you will permit the paradox, almost too useful to be formulated. As the association grew, both comparative anatomy, mammalian embryology, and physical anthropology were brought in to broaden the field of work and widen the scope of interest.

Through embryology the common origin of organs and systems is revealed and the form relations of the adult explained. Comparative anatomy has shown the correlation of form with function, and by it the phylogenetic relationships of man have been made evident.

But aside from advances of the sort just cited, the chief virtue of this more generous interpretation of anatomy has been to emphasize the fact that the animal body is in a continual state of flux and change, even from the structural standpoint.

The moment attention is directed to the fact of change—be it ontogenetic or phylogenetic—the inevitable tendency is to endeavor to determine how such change occurs—and this leads to experimental work with the attempt to relate the character of the process to the details of the accompanying structural alteration. Fortunately studies which include experimental

modifications have been welcomed at our meetings. The analysis of the programs shows an average of 14 per cent of experimental papers up to 1910 and from that date on, an average of 32 per cent—a very notable increase.

We shall agree, I believe, that a broad policy should be maintained and the presentation of work of this character encouraged.

Such an attitude, however, desirable as it may be, does not meet our needs completely. It is also important for us to push further on in the direction in which these first steps have been taken, and I regard this occasion as an opportunity to say something concerning the course and character of this coming effort.

We shall all admit that the ultimate problems of biology lie in the field of function. The investigator aims to control, to explain and to foretell activities, and in this connection it is fitting to recall that it comes down to us from Aristotle that the most important question concerning an animal is how it lives—not what it looks like.

The functional phenomena are naturally the most important, first because of our deep interest in the dynamics of human life, and second because as we progress in the study of function, we pass from the cell—the unit of structure—to the more precise units of chemical activity, and these latter studies are thus made in the terms of the most fundamental masses yet recognized.

When functional processes are expressed by a formula, the animal sometimes appears a bit superfluous, but a description of the structures involved is generally required to complete the picture.

All physiological reactions imply structure—for the cells are the loci of the activity. To be sure only a portion of the structural peculiarities of cells is ever visible, but when studying the activities of an animal or its organs or tissues, it is of fundamental importance for us to possess the most detailed information available concerning the structural make up of that which is being tested.

Thus interest in function normally carries with it a like interest in structure, and conversely structures which have known

functions become those worthy of the most intense study from the standpoint of anatomy.

Apropos of this aspect of the question before us, permit me a word on the subject of general physiology. The study intended to discover the laws of living substance, wherever found, was well started on the continent in the early seventies—but just at that time the many problems in morphology and phylogeny brought into prominence by the work of Darwin, diverted the young men of the period—and nearly a generation had to pass before the subject was again taken up and developed as we find it today. For us the interest of this earlier endeavor lies in the fact that it was based on a sound knowledge of structure and represented the sort of appreciation of the inter-relations of form and function—for which I would here make a plea.

Of course, since anatomy and physiology received their present elaboration, it hardly has been possible for individual workers to command, even in limited fields, either the experience or the information that would make their results equally valuable from both points of view. In a sense this state of affairs has come to stay—but on the other hand it is mitigated by the fact that scientific progress is accompanied by something more than the heaping up of many details. Definite conclusions emerge and generalizations are established, so that considered as usable information, the accumulated data gradually become more and more available for those not immediately occupied with the initial experiments or observations.

As students of structure it is important for us to utilize the physiological information thus prepared, for in many cases it gives the significance to structural features which is needed to make those features intelligible.

Despite the close interdependence of form and function, there often appears a curious antagonism between the workers in these two fields, an antagonism that amounts almost to a contempt for the kind of work done by the other man. When each is following his own line narrowly—be he concerned with function or with structure—I am not sure but that the antagonism is justified. Yet all work is not necessarily as restricted

as the conventional labels might suggest, and so far as any work in either field is broad, it is not open to hostile criticism solely by reason of its major content.

If then, we break through historical limitations, and as anatomists extend our interest to the entire vertebrate series—including in the case of each form everything between the germ cells and the senile animal, and if in our descriptions we recognize functional phases which are more or less under control, and on occasion exercise such control, we have marked out for study a field which includes all of the general biological problems, and which also may be approached from the angle of our special interest.

Speaking in concrete terms we may put the case as follows: Given an animal the biological questions which it raises can be enumerated thus:

Its systematic position; its place in the palaeontological series; its distribution; its ecology; its behavior; its heredity; its functions in the terms of tissues and organs—including the chemistry of the energy interchanges; its structural features from the ovum to the senile animal, in terms not only of gross anatomy, but also in the terms of constituent cells and cell structure, as well as in physical or chemical terms. These represent the concrete problems while over and above them floats the great cloud of general questions.

The answers to all the questions thus indicated are to be sought with the idea of comparing the data gathered from the form examined with those gathered from other forms, for the purpose of obtaining general conclusions. Such a program calls for a good deal of downright description.

Every biological investigation falls somewhere within this general frame, but in each instance has its own peculiar affiliations and its special goal.

If we turn now to the question of the direction in which our work as an association might move with advantage—it is necessary to clear the ground a bit before passing to particulars.

In the first place it is self evident that biological work is bound to produce results applicable to man—and these results will

some time or other be added to our store of common knowledge. All our work moves that way just as steadily as our solar system moves towards the constellation of Lyra—though unfortunately not so fast. This definite trend need not worry us, for it has no bearing on what we sometimes call pure science.

Pure science is the work of those who endeavor, as they go, to clear up the underlying problems brought to light by their special studies, rather than to press the immediate application of crude results. The practice is largely a question of temperament, and the definition one of degree.

We have seen that traditional anatomy has been vivified by the inclusion of comparative anatomy, physical anthropology and embryology—all of them still very live subjects—but this is no reason why we should not look about for other fields which might also be cultivated with advantage.

Doubtless each of us could present a list of biological topics that might be added to those already enumerated—and it is probable that such lists, while they would largely agree, would always express something of the personal preoccupations of the men who made them.

Thus, were I asked to name some directions in which we might extend our work I should naturally lay weight on post-natal growth in the terms of cell multiplication and cell structure, with its many subsidiary problems, and also on the need for more precise information touching the chemical constitution of tissues and organs as modified by advancing age—for these changes must be of prime importance from the standpoint of function. This illustrates my point. Details in this direction are however not in place at this time, but it is perhaps worth while to emphasize the value to each one of us of formulating with some exactness for himself the general lines of work which might be followed—and of determining their relations to each other—whatever his particular program may be—for when an investigation is to be carried out, these formulations offer the investigator something solid on which he can lay hold.

The question of the direction in which one should move is intimately related to the larger question of what things are worth

while. A common answer to this latter question is that one cannot tell what will be worth while—but I wonder whether this agnostic attitude is quite justified.

If we look on science as coöperative, it follows that our individual work should have some recognizable relation to the larger problems or special interests of our own time, and the worth of our personal activity can, in one way, be measured by the degree to which the worker himself can link with his own activities those of his contemporaries.

I put the statement in this form because others may not at once see the significance of an investigator's work and may even require to have it hammered in, but if the man himself sees it, and in the end can make it visible, he has arrived.

This problem of worth links itself closely with the broad question of the way in which the body of knowledge constituting any science is built up; the question of "the work that lives."

The answer to this last question is simple. The work that lives is that which is useful to other investigators—whether it be an hypothesis, a method or an observation. Other work no matter how brilliant in itself or how remarkable the technical abilities were that lead to its accomplishment, has but an ephemeral existence.

There is on the other hand a sort of ferment action exercised by thorough researches, for when an investigator reaches the point where he is able to offer generalizations, he specially contributes to the advancement of learning, for it follows as one result of his activity that his colleagues will be fairly certain to hunt assiduously for weak points in his work, and thus he acquires in the scientific world the honorable position of an organizer of labor.

If you will look over the titles of any list of classical memoirs in science which have been thought important enough to be reprinted, you will find not only in biology, but in the whole realm of natural science, the sort of work to which I would direct attention.

It is of interest to note that among the investigations thus honored, there is no stereotyped work, characterized by the

accumulation of results without improvements in method, or studies with a time clock attachment or investigations that are prescribed.

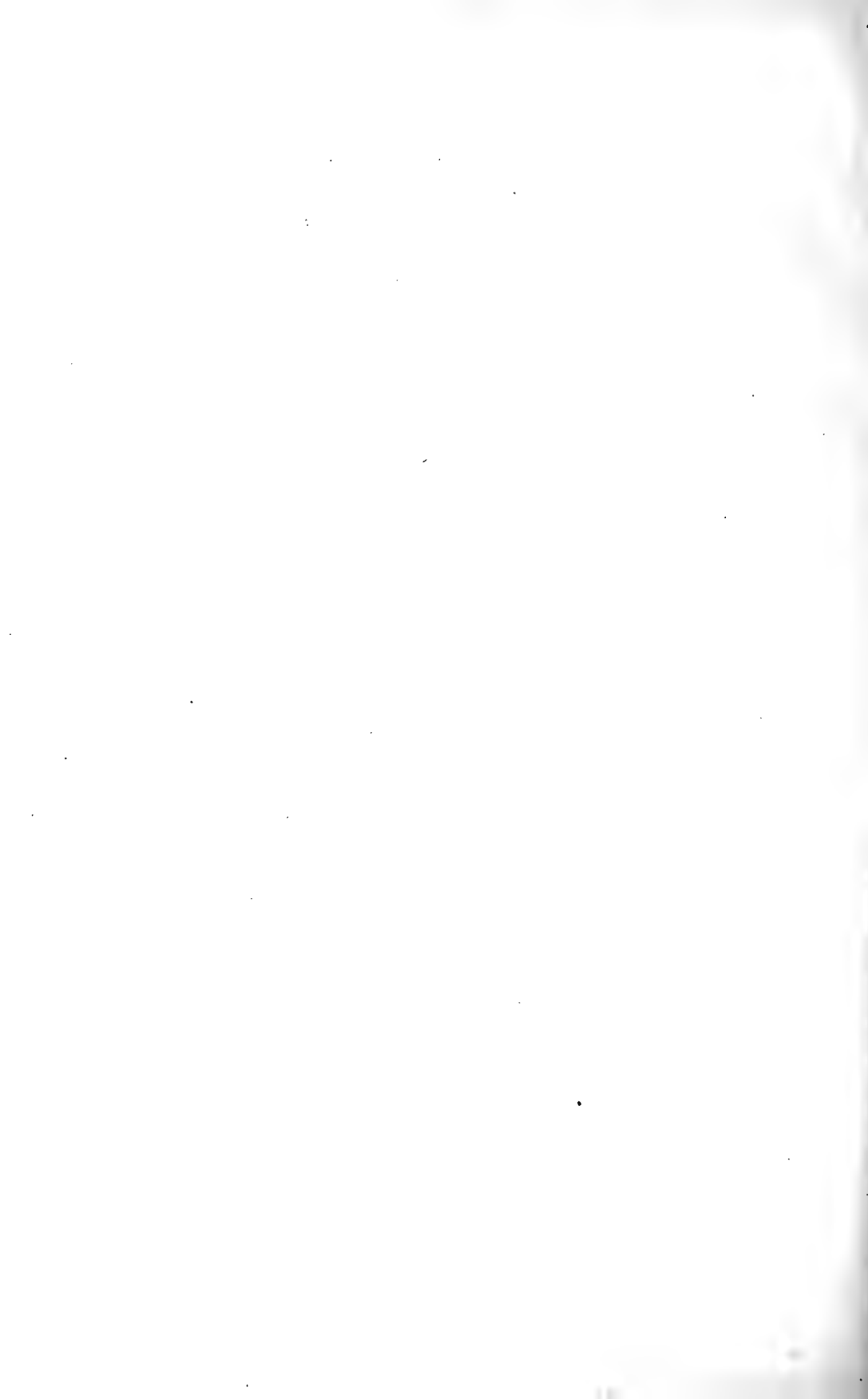
The moral appears to be that the effective investigator is a free man: Yet along with this essential freedom must go limitations which are self imposed. As there is no such thing as an isolated idea it follows that in the world of thought it is possible to start from any point and by successive steps of association pass to any other point whatsoever. The investigator is continually called upon therefore to curb his interests or rather his activities—while pushing forward towards some goal which he himself clearly perceives. Thus it happens that the mature research always appears framed and oriented, and the author takes pains to inform us of the direction in which he is moving.

Each fundamental science has at its core a few more or less precise concepts in relation to which it grows. A derived science like biology also has its own central concepts. To add to these concepts or to modify them by extension or rectification is to contribute. To this end descriptive and technical work are both important and necessary, but in the case of biology, there is also the functional side which the student of structure needs to appreciate. Something is gained if he admits it is there, but more if he takes it into serious consideration.

I conclude therefore that although we are entrusted with the grave responsibility of preserving and transmitting anatomical knowledge, and are designated by the ancient and honorable title of Anatomist, yet we are at liberty to reject anything historically implied by that title which might prove hampering to our present work. Nevertheless our group is represented by those trained primarily in the study of structure and such studies must remain our chief occupation.

It is not possible however to contribute effectively to the solution of the larger biological problems unless the functional responses associated with structures are kept clearly in view—or even examined.

So long then as animals or their parts are studied comprehensively by us, we may hope to keep intellectually alert, and it is my conviction that our scientific worth as an association will in the main depend on the persistence with which we follow an inclusive plan of investigation and maintain the broader view.



PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

THIRTY-THIRD SESSION

*Anatomical Laboratories of Cornell University, New York
University and Columbia University, New York City,
December 27, 28 and 29, 1916*

WEDNESDAY, DECEMBER 27, 9.00 A.M.

The thirty-third session of the American Association of Anatomists was called to order by President Henry H. Donaldson, who appointed the following committees:

Committee on Nominations for 1917: J. P. McMurrich, chairman; G. Carl Huber, George A. Piersol.

Auditing Committee: F. T. Lewis, chairman; S. W. Ranson.

The morning session for the reading of papers concluded with an address by the president, Prof. Henry H. Donaldson, on "Biological Problems and the American Association of Anatomists."

THURSDAY, 12.30 P.M. ASSOCIATION BUSINESS MEETING, PRESIDENT HENRY H. DONALDSON, PRESIDING.

The Secretary reported that the minutes of the Thirty-second Session were printed in full in *The Anatomical Record*, volume 10, number 3, pages 133 to 269, and asked whether the Association desired to have the minutes read as printed. On motion, seconded and carried, the minutes of the Thirty-second Session were approved by the Association as printed in *The Anatomical Record*.

Prof. F. T. Lewis reported for the Auditing Committee as follows: The undersigned Auditing Committee has examined the accounts of Dr. Charles R. Stockard, Secretary-Treasurer of the American Association of Anatomists and finds the same to be

correct with proper vouchers for expenditures and bank balance on December 23, 1916, of \$264.34. (Signed) F. T. LEWIS, S. W. RANSON.

The Treasurer made the following report for the year 1916:

Balance on hand December 21, 1915, when accounts were last audited.....	\$264.09	
Receipts from dues 1916.....	2222.88	
		<hr/>
Total deposits.....		\$2486.97
Expenditures for 1916:		
Postage and telegrams.....	\$40.55	
Printing and stationery.....	68.15	
Collection and exchange.....	2.53	
Expenses of Secretary-Treasurer, New Haven Meeting...	17.80	
Wistar Institute for subscriptions Journal of Anatomy, Anatomical Record, etc.....	2064.00	
Stenography-typewriting.....	29.60	
		<hr/>
Total expenditures.....	2222.63	
		<hr/>
Balance on hand.....		\$264.34
Balance on hand, deposited in the name of the American Association of Anatomists in the Corn Exchange Bank, New York City.		

On motion the reports of the Auditing Committee and the Treasurer were accepted and adopted.

The Secretary announced that the Committee on Nominations, through its Chairman, Prof. R. R. Bensley, places before the Association the following names: For members on the Executive Committee, terms expiring in 1920, Prof. Franklin P. Mall and Prof. James Playfair McMurrich.

On motion the Secretary was instructed to cast a ballot for the election of the above named officers.

The Secretary presented the following names recommended by the Executive Committee for election to membership in the American Association of Anatomists.

- ALLEN, ERZA, A.M., Ph.D., Professor of Biology, *Philadelphia School of Pedagogy*, 125 Thompson Ave., Ardmore, Pa.
- AMSBROUGH, A. E., A.B., Student of Medicine, *University of California, Berkeley, Calif.*
- BAILEY, PERCIVAL, B.S., Assistant in Anatomy, *Northwestern University Medical School, Chicago, Ill.*

- BYRNES, CHARLES M., B.S., M.D., Instructor in Neurology, *Johns Hopkins Medical School, 207 E. Preston St., Baltimore, Md.*
- CAREY, EBEN J., Instructor in Anatomy, *Creighton University Medical Department, Omaha, Neb.*
- CARVER, GAIL L., A.B., A.M., Professor of Biology, *Mercer University, Macon, Ga.*
- CUMMINS, HAROLD, A.B., Instructor in Histology and Embryology, *Vanderbilt University Medical School, Nashville, Tenn.*
- DUBREUIL, G., M.D., Professor of Anatomy, *Institut d' Anatomie, Universite de Bordeaux, Bordeaux, France.*
- EATON, PAUL BARNES, A.B., M.D., 1306 W. Lexington St., Baltimore, Md.
- FISHER, HOMER G., A.M., Student, *Johns Hopkins Medical School, Baltimore, Md.*
- GEE, WILSON, M. A., Ph.D., Professor of Biology, *Emory University, Oxford, Ga.*
- GIBSON, G. H., M.D., *Waitangi, Chatham Islands, Wellington, New Zealand.*
- HOLT, CAROLINE M., A.B., Ph.D., Assistant Professor of Biology, *Simmons College, Boston, Mass.*
- JOHNSON, SYDNEY E., Ph.D., Instructor in Anatomy, *Northwestern University Medical School, Chicago, Ill.*
- KEEGAN, JOHN J., A.M., M.D., Instructor in Anatomy, *University of Nebraska Medical College, Omaha, Neb.*
- KOCH, JOHN C., B.A., Student of Medicine, *Johns Hopkins Medical School, Baltimore, Md.*
- KUNITOMO, KANAE, M.D., Professor of Anatomy, *Nagasaki Medical School, Nagasaki, Japan.*
- LATIMER, HOMER B., A.M., Associate Professor of Zoölogy, University of Nebraska, 1909 South 27th Street, Lincoln, Neb.
- LEWIS, MARGARET REED, M.A., Collaborator, Department of Embryology, Carnegie Institution of Washington, *Johns Hopkins Medical School, Baltimore, Md.*
- MORRIS, MARGARET, B.A., Ph.D., *Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*
- MURRAY, H. A., JR., A.B., Student, Columbia University, College of Physicians and Surgeons, 437 West 59th Street, New York City.
- NORRIS, EDGAR H., B.S., A.M., Assistant in Anatomy, *University of Minnesota, Minneapolis, Minn.*
- PFEIFFER, JOHN A. F., M.A., M.D., Senior Asst. Physician and Pathologist, *Government Hospital for the Insane, Washington, D. C.*
- RASMUSSEN, ANDREW T., A.B., Ph.D., Instructor in Neurology, *University of Minnesota, Minneapolis, Minn.*
- RINGOEN, ADOLPH R., Assistant in the Department of Animal Biology, *University of Minnesota, Minneapolis, Minn.*
- ROBERTSON, ALBERT DUNCAN, B.A., Professor of Biology, *Western University, London, Ontario, Canada.*
- ROSE, FRANK H., A.B., Austin Teaching Fellow, *Harvard Medical School, Boston, Mass.*
- SCHULTZ, ADOLPH H., Ph.D., Collaborator in Embryology, Carnegie Institution, *Johns Hopkins Medical School, Baltimore, Md.*
- SHARP, CLAYTON, A.B., M.D., Instructor in Anatomy, Columbia University, College of Physicians and Surgeons, New York City.

SMITH, H. P., A.B., Student of Medicine, *University of California, Berkeley, Calif.*
SMITH, WILBUR CLELAND, M.D., Assistant Professor of Anatomy, *Tulane University, New Orleans, La.*

SWINDLE, GAYLORD, Ph.D., Instructor in Anatomy, *Washington University Medical School, St. Louis, Mo.*

TURNER, C. L., B.A., M.A., Instructor in the Department of Anatomy and Biology, *Marquette University School of Medicine, Milwaukee, Wis.*

WHEELDON, THOMAS FOSTER, A.B., A.M., Austin Teaching Fellow, Department of Anatomy, *Harvard Medical School, Boston, Mass.*

WHITTENBORG, A. H., M.D., Professor of Gross-Anatomy, *College of Medicine, University of Tennessee, Memphis, Tenn.*

WILLIAMS, JAMES WILLARD, B.A., M.A., Professor of Biology, College of Yale in China, Changsha, China. (*Care of G. H. Malone, Nanking, China.*)

On motion, the Secretary was instructed to cast a ballot for all the candidates proposed by the Executive Committee. Carried.

Motion was made by Prof. S. W. Ranson, that a committee of three be appointed by the President to consider the matter of nomenclature relating to the Sympathetic Nervous System. Seconded and carried.

The Association passed the following resolution regarding the National Research Council:

Be it resolved: that the American Association of Anatomists hereby registers its approval of the coördination and federation of the research agencies in the country undertaken by the National Academy of Sciences, and expresses its willingness to join with and assist the National Academy in accomplishing the above federation.

In connection with this, it was further moved by Professor Harrison that the Association coöperate with the Committee of One Hundred on Research and the National Research Council and that the Chair designate a Committee to work jointly with other members appointed by the Committee of One Hundred and the National Research Council. Seconded and carried.

On motion the Association adjourned.

FRIDAY, DECEMBER 29. A SHORT BUSINESS SESSION FOLLOWED THE SCIENTIFIC SESSION.

President Donaldson announced the appointment of the following committee to consider the matter of nomenclature relat-

ing to the Sympathetic Nervous System—Prof. G. Carl Huber, chairman; Profs. S. Walter Ranson and Irving Hardesty.

It was then moved and voted that the Association express through the Secretary appreciation of the valuable aid The Wistar Institute is rendering the Association and the progress of anatomy in this country by its most generous and efficient publication of the Anatomical journals. Further The Wistar Institute has contributed greatly to the success of the present and past two meetings by its prompt publication and distribution of the abstracts of the communications presented.

It was moved and voted that the Association express through the Secretary its thanks and appreciation to Cornell University Medical College, New York University and Columbia University for the cordial hospitality and the manner in which the Association has been accommodated and entertained.

CHARLES R. STOCKARD,

Secretary of the Thirty-Third Session of
the American Association of Anatomists

ABSTRACTS OF PAPERS
PRESENTED AT THE
THIRTY-THIRD SESSION
OF
THE AMERICAN ASSOCIATION OF ANATOMISTS
DECEMBER 27, 28 AND 29, 1916
AT
NEW YORK CITY

(All papers marked with an asterisk (*) were read only by title)

1. *The Golgi apparatus in the cells of the distal glandular portion of the hypophysis.* WILLIAM H. F. ADDISON, University of Pennsylvania.

The Golgi apparatus in the cells of the distal glandular portion (pars anterior) of the hypophysis of the albino rats is demonstrable by several methods. It is shown in thin sections ($3-4\mu$) in the basophilic and acidophilic cells. After castration the basophilic cells undergo distinct changes (Anat. Rec., January, 1916) but the Golgi apparatus persists throughout these changes, apparently functioning as a definite cell organ.

The methods used have been the neutral formol-bichromate-sublimate fixation of Bensley, (Biol. Bull., 1910), the neutral formol-bichromate procedure of Cowdry, as well as the special methods—the osmic acid method of Kopsch, and the method of Golgi as given by Kulesch (Arch. f. Mikr. Anat., 1914) and Riquier (Arch. f. Mikr. Anat., 1910). The pictures of the Golgi apparatus given by these several methods are quite comparable although of very different appearance. In the preparations made by the Bensley or Cowdry fixations, the general appearance of the Golgi apparatus may be studied after the usual staining methods—Mallory's aniline blue-orange G., iron hematoxylin, or hematoxylin and eosin. It is of larger size and so is more readily seen in the large basophilic cells. Here it shows as a round or oval condensed spot, surrounded by a lighter ring, situated within the cytoplasm near the nucleus. The surrounding lighter zone is no doubt accentuated by

shrinkage due to difference in density between the substance of the Golgi apparatus and the surrounding cytoplasm. In a three-month normal rat hypophysis, prepared by one of these methods, the basophiles measure $14\mu \times 11\mu$, with nuclei $6.7\mu \times 5\mu$. Here the Golgi apparatus measures on the average $5.5\mu \times 3.8\mu$. In three-month animals which had been castrated two months previously, the basophiles measured $19\mu \times 15.5\mu$ and the Golgi apparatus $8\mu \times 5\mu$. Thus with the increase in size of the basophiles, the Golgi apparatus has also increased. In the basophilic cells of animals which had been castrated more than two months, large vacuoles developed. These vacuoles press aside the nucleus and the Golgi apparatus, but the latter retains its definite structure although often somewhat flattened by the enlarging vacuole. In the acidophilic cells, which are smaller in size than the basophiles, the Golgi apparatus is also smaller and hence not so conspicuous. Gemelli, an early observer (Boll. Soc. Med. Chir. Pavia, 1903) pictures much larger structures, which extend as a network throughout a considerable portion of the cytoplasm, but his experimental animal and technique were different.

With the silver methods of Golgi the deposits of silver are within the spots. Often they show as a localized condensed mass but in other parts of the same preparation the black deposit takes on a network aspect. With the osmic acid method of Kopsch, the network appearance is often more sharply defined. What the nature of this appearance may be, is at present a topic of debate, but the fact that it persists when the cell is undergoing cytomorphic changes may be put forward as evidence that it is here a definite cell organ.

2. *The behavior of the interstitial cells of the testis towards vital dyes.*

WILLIAM H. F. ADDISON and J. MONROE THORINGTON, University of Pennsylvania.

On examining sections of testes of animals (*e.g.*, white mice and white rats) which had been previously injected with trypan blue, distinct scattered blue spots are seen between the seminiferous tubules. This appearance was interpreted by Goldmann ('09) as being that the interstitial cells of Leydig had collected the blue within themselves. From our studies it would seem that the blue is not within the glandular interstitial cells, but within other cells, which are of connective tissue types, and which normally compose part of the intertubular cell-masses.

Before Bouin and Ancel ('03 and later) brought forward strong evidence for the hypothesis that the interstitial cells of the testis represented a gland of internal secretion, these cells were regarded as trophic structures, with the function of passing on nutrient material from the blood vessels to the various cells within the seminiferous tubules. In this latter view, Goldmann concurred and believed that he could follow the processes of the interstitial cells forcing their way into the tubules and so purveying granular nutritive substances directly either to the Sertoli cells or to the spermatids. H. M. Evans (Science, '14)

found "in the testis the interstitial cells of Leydig are stained brilliantly although the granules here are singularly regular and intermediate in size between those possessed by cells of types one and two in the skin. In addition, true vitally stained connective tissue cells of type two are present between the seminiferous ducts."

In our series of experiments on male white mice and white rats, in which trypan blue was injected subcutaneously, the reaction to the stain was typical. When thin sections ($3-5\ \mu$) of the testes were examined, the blue coloration was seen to be confined within a small number (2-3-6) of cells in each intertubular mass. These cell-masses are often triangular in shape, as seen in cross-section of the testes, and the blue-containing cells were disposed singly and situated, for the most part, at the periphery of, and often one at a corner of the intertubular cell-masses. By counterstaining it was seen that the glandular interstitial cells were free from the dye. The cells containing the blue were of two appearances. Some were elongated in the form of fibroblasts, but the greater number were rounded or polyhedral. These latter from their finely vacuolated cytoplasm and often slightly irregular oval nucleus, might be regarded as clasmatoocytes. The nuclei of these were smaller and of more homogeneous appearance than the nuclei of the glandular interstitial cells. According to Evans' definition, both types of cells containing blue would be included under the term macrophage.

Thus by the use of trypan blue it is possible to distinguish between glandular interstitial cells and macrophages in the intertubular cell-masses of the testes of mice and rats.

3. *On the origin and fate of the osteoclasts (lantern).* LESLIE B. AREY, Northwestern University Medical School.

Since their discovery by Robin in 1849, the polykaryocytes of developing bone have been regarded commonly as the agents of bone resorption. For this reason these multinucleate cells have been termed 'osteoclasts.'

Views as to the origin of the osteoclasts are not in accord. Kölliker maintains that they arise from osteoblasts by repeated nuclear division; Howell infers an origin by osteoblastic coalescence; Bredichin derives them from fused bone cells; Ranvier, Duval, and Böhm from lymphoid marrow cells; Mallory from fused endothelial leucocytes; Wegener and Schaffer from the endothelium of capillaries; Kaczander from cartilage cells. The results of Jackson, Danchakoff and Maximow agree in tracing the origin of the *first* osteoclasts in the early stage of bone development to enlarged reticular cells of the bone marrow. These cells possess at first but two or three nuclei and the cytoplasm is basophilic. Later their cytoplasm becomes oxyphilic and the nuclei may become extremely numerous.

A variance of opinion exists also as to the resorptive potentialities of the osteoclasts and the manner in which the nuclei increase in number. According to Kölliker and Jackson these cells actively resorb

the bone matrix, while the number of nuclei is increased by nuclear division. Bredichin views the osteoclasts as transitional stages in the transformation of bone matrix into marrow and granulation tissue, with a coincident multiplication of the nuclei of the component bone cells. Danchakoff speaks of the confluence of mesenchymal cells. Maximow believes that large osteoclasts arise at the expense of smaller ones, the multinuclear cell-masses thus formed being ameboid phagocytes. He never observed nuclear division either by mitosis or amitosis. F. T. Lewis emphasizes the absence of direct evidence respecting a resorptive activity on the part of the osteoclasts and rejects their origin by cell fusion. They are rather to be regarded as degenerating cells "produced by those conditions which lead to the dissolution of bone."

Concerning the ultimate fate of the osteoclasts, Kölliker believed that they might resume an osteoblastic function after their resorptive activity had ceased. Jackson rejects this view and maintains that both osteoclasts and bone cells return to a reticulum similar to that from which he holds they took origin. Maximow differs with Jackson in that some osteoclasts are said to be destroyed through extreme degeneration.

The untimely death of Prof. C. W. Prentiss interrupted an investigation which he had been pursuing on the origin and fate of the osteoclasts, and relative to which he had published a brief note. A reinvestigation and extension of these observations, made by the writer, form the basis of the present preliminary communication. Observations have been made on membrane-bone of human, and especially of pig embryos. A favorable site for study is found about the walls of the dental alveoli where active bone resorption is preparing for the accommodation of the rapidly growing teeth. Here osteoclasts appear in large numbers.

In regions where bone is actively forming the osteoclasts are columnar and distinct with basophilic cytoplasm. During development the cytoplasm diminishes in amount, and in older regions the still basophilic osteoblasts flatten out and form syncytial masses. While the osteoclasts may arise from reticular cells in the early stages of bone development, my observations indicate that in later stages they take origin from the osteoblastic syncytia just described. There were found all transitional stages between these syncytia with basophilic cytoplasm, staining blue with hemotoxylin, and typical oxyphilic osteoclasts staining red with eosin. Furthermore, osteoclasts were seen frequently continuous at either end with basophilic osteoblasts and particularly with osteoblastic syncytia.

According to these observations, therefore, the osteoclasts arise from depleted osteoblasts which have first formed a syncytium before being transformed into the oxyphilic osteoclasts. Nuclear division by mitosis or amitosis has never been observed, although mitoses were not uncommon in the nearby germinative layer of the epidermis. As bone resorption continues, new osteoblasts come into relation with

the osteoclasts and are incorporated into them, so that in general, the larger an osteoclast, the more numerous its nuclei and the more extensive its probable bone resorptive history.

But the osteoblasts, as such, are not the only source from which the nuclei of the osteoclasts are recruited. Bone cells, embedded in the matrix, are laid bare by the resorptive processes and are ingested by the osteoclasts following in the wake. All intermediate conditions may be found between the initial and final stages of inclusion. Furthermore, bone cells are surrounded normally by a capsule which resists the action of strong hydrochloric acid. Encapsulated and distinctly stellate cells, which resemble bone cells, may occasionally be found embedded in the osteoclastic cytoplasm. Such cells are interpreted as bone cells whose capsules are still resistant to cytoplasmic digestion. From the relative infrequency with which persistent capsules are seen it is probable that the enclosed bone cells are eventually liberated. Ingested bone cells must contribute in substantial numbers to the formation of osteoclasts.

Here it appears that the degree of multinuclearity is an index of the number of osteoblasts and bone cells entering into the composition of the osteoclasts.

There is no direct evidence as to how bone matrix (inorganic and organic) is resorbed. One may assume that it is essentially a process of decalcification and digestion through the agency of an acid and an enzyme. The relation of the osteoclasts to these changes can only be inferred. The facts that osteoclasts are plentiful where resorption is going on and disappear when resorption ceases, that they are applied closely to the surface of the bone, that they often wrap themselves around irregular spicule-processes or lie in distinct pits (Howship's lacunae), that the surface applied to the bone possesses at times a striate border, and that the staining reaction differs from osteoblasts, all suggest but do not prove a resorptive activity.

As to the fate of osteoclasts. That these cells may be resolved finally into osteoblasts and again act as bone-formers seems improbable. My preparations show nothing in favor of such a cycle, whereas pictures of degeneration in varying degrees are abundant. The onset of cytoplasmic degeneration before the cessation of bone-resorptive activity seems to be not uncommon. At times degenerating osteoclasts, singly or in nests, are found stranded in the marrow tissue. These often exhibit extreme vacuolization and other degenerative changes. To a certain extent they probably atrophy and disappear although indications of a transformation into marrow reticulum are not lacking. Large osteoclasts have been observed rarely in the blood vessels of the bone marrow. That such gain admittance, and do not arise *in situ* is supported by their degenerate appearance, due to cytoplasmic vacuolization, granular degeneration and pyknotic nuclei.

Summary. 1. While osteoclasts may form in the early stages of bone development from reticular cells of the marrow, in later stages they arise from syncytia of depleted osteoblasts.

2. The numerous nuclei of large osteoclasts are derived (a) from the constituent osteoblasts, and (b) from bone cells which are ingested as the bone matrix is resorbed.

3. Only indirect evidence points to the osteoclasts as the active agents in bone-resorption; they may also be interpreted as degenerating osteoblasts.

4. Eventually the osteoclasts either atrophy and disappear or are resolved into the reticulum of bone marrow.

4. *On the relation between neural and intermediate portions of the hypophysis.* (Lantern.) WAYNE J. ATWELL, Department of Anatomy, University of Michigan.

In a study of the development of the hypophysis in the rabbit an intimate association of neural portion and intermediate lobe has been observed. This union of the two parts is first seen in rabbit embryos of fifteen days' development. Not all embryos of this day present the phenomenon, but every sixteen days' embryo examined shows one or more definitely circumscribed regions in which the two parts are in very intimate relation.

The conditions found in rabbit 8 A (sixteen day embryo; 5 μ series) may be taken as typical. The hypophysis region of this embryo was carefully reconstructed in wax and then the neural and intermediate parts were separated. On viewing the model of the neural lobe from the surface which was in apposition to the intermediate part, four distinct areas of contact may be seen. They are arranged in two pairs, one of which is near the caudal, or free, end of the lobe. The other pair lies about midway between the free end of the lobe and the place of its attachment to the brain wall. The combined areas of these four contact regions approximates one-third of the entire surface of the lobe facing the intermediate portion. One of the caudal areas is the largest of the four and no two are of the same size. Embryos which present only one region of contact have that one near the caudal end of the lobe.

From sections it may be seen that the basement membranes of the two parts are lacking at the places of contact. For this reason it is not easy to determine whether the contact is due to the active ingrowth of one part into the other or merely to the passive fusion of the two parts. There seems to be considerable evidence, however, from staining reactions and from the arrangement of cells that the contact is due to the active penetration of the neural portion into the intermediate portion. These contacts, or ingrowths, are, of course, not to be confused with the growth of connective tissue into the gland, which takes place, in the intermediate lobe, about the nineteenth or twentieth day of development.

While every sixteen-day embryo examined presents at least one region of contact, older embryos do not all show them. In certain eighteen, nineteen and twenty-day embryos the two portions of the hypophysis are entirely separate, each showing its own, uninterrupted

basement membrane. Such of the older embryos as do show contact regions have them reduced both in size and in number.

One further observation should be here recorded. On the surface of the intermediate part facing the residual lumen of Rathke's pocket may be seen a slight depression to correspond with the center of each contact area found on the opposite surface of the part.

In attempting to interpret the above-recorded observations for the rabbit the following possibilities present themselves:

1. The contacts or ingrowths may be an attempted ontogenetic repetition of the condition which is normal for certain fishes.

2. An opportunity is offered for the early intermingling of elements of neural and intermediate portions of the hypophysis. On this account, and because of the conflicting statements concerning the adult structure of the part, a careful study of the histogenesis of the intermediate lobe in mammals is very much to be desired.

5. *Studies of the cortex of the sheep brain.* CHARLES BAGLEY, Jr., Phipps Psychiatric Clinic, Johns Hopkins University.

The purpose of this investigation was to outline clearly the cell and fiber architecture of the sheep brain, with the hope of establishing a foundation for further experiments.

The brain of the sheep was selected for the following reasons: 1) Those interested in cerebral localization have scarcely touched upon the whole group of ungulates and there has been no attempt made to present a complete study of the brain of this member of the group. 2) To facilitate the use of the brain in teaching the anatomy of the central nervous system. 3) The animal lends itself well for experimental study, is easily obtained and can be cared for with but little more difficulty than the more common laboratory animals. 4) The specific starting point was von Monakow's claim of the great size of the frontal lobe and its relation to the red nucleus—a contention which we soon found untenable.

Methods. The plan of attack has been: (a) the study of the brain in phases of development; (b) survey of the cell and fiber structure of the normal adult brain, and (c) experimental ablation.

For the embryological studies serial sections were made of brains of embryos varying from 2 to 47 cm., the latter being about full term.

The architecture of the normal adult brain was first studied in a series of frontal sections of the brain and later in a series of sections made after individual dissection of the gyri—the latter having the advantage of easy orientation and accurate right angle cutting of the laminae.

Experimental ablation of areas previously outlined histologically was done in five lambs (three to five weeks). These brains are now being sectioned and will be stained by the Pal-Weigert method for the study of fiber changes and with various cell stains, to demonstrate alteration in the nuclei of the brain-stem.

Observations. In the embryological series we have outlined the order of development of the sulci and gyri, the period at which cell differentiation is sufficient to present lamination in various portions of the cortex, and the order of myelinization.

The studies of lamination demonstrate five principal areas, connecting which there are narrow zones of cortex of transitional type. The fiber structure also permits of division into areas, but to a less definite degree.

The results of the ablation experiments cannot be stated until further work is done on adult brains, followed by Marchi stains.

*6. *The effect of ultra-violet light rays upon the development of the frog's egg: (2) the artificial production of folded (u-shaped) embryos.* W. M. BALDWIN, Albany Medical College (Union University).

This paper is the second of a series on the effects of radiation on certain areas of the fertilized and undivided ovum of the frog by means of ultra-violet light rays. The source of energy utilized was an electric arc provided with iron electrodes and actuated by a high-frequency direct current. The illumination of small surface areas of the eggs was brought about by the use of a perforated tinfoil diaphragm. The eggs measured 1.7 mm. in diameter, while the perforations varied from 0.3 to 0.4 mm. in diameter. Consequently, but a comparatively small surface area was influenced in any one experiment. The intensity of radiation was such that an exposure of from twenty to thirty seconds was sufficient to produce developmental defects. The area studied by this method and reported in this paper extended from the region of the equator up to the animal pole, but was exclusive of the pole itself and of a narrow median strip connecting this pole with the equator. The developmental end-product was, in every instance, what is best described as a U-shaped embryo of that general type in which the two folded body-parts lay upon the same horizontal plane.

A singular result of the study of the sectioned specimens, in addition to the striking feature of the uniformity of production of this type of deformity, is the absence of defects in the anatomy of the developed tadpole. Comparatively little has been ascertained concerning the chemical effect brought about by the action of the rays upon the egg mass. Notwithstanding, the conclusion was drawn from studies upon these defective eggs during the process of development that the chemical composition of the cell-mass thus radiated was so altered that it lagged behind in what might be termed the chemical development of the ovum and though ultimately participating in the developmental processes exercised a retarding influence upon some of the gross mechanical shiftings of embryonic anlage. The altered mass, located as it was lateral to the definitive median plane of the embryo, retarded, in the first place, the medianward shifting of the neural tube-half on its own side and induced, in the second place, an exaggerated migration of the opposite tube-half medianward and beyond, towards the affected area, before coaptation with the neural tube-half of that side with the production of a whole tube could be brought about.

7. *The development of the serous glands (von Ebner's) of the vallate papillae in man.* E. A. BAUMGARTNER, Washington University Medical School.

Serial sections of the vallate papillae in fetuses and new-born form the basis for this paper. Wax reconstructions of the papillae in the smaller fetuses were made and studied.

In a reconstruction from an 8.5 cm. fetus (crown-rump length) the epithelial down-growth forming the limits of the papilla is complete; growing inward from this are three well defined solid bodies, the early gland anlagen, together with less well defined outgrowths. These gland anlagen in a 10 cm. fetus present enlarged ends and slightly constricted stalks, the beginnings of terminal acini and ducts of later stages. The papilla in front of the foramen caecum has many glands extending both from the lower margin and lateral walls of the circumscribing epithelium; at the caudal end of the papilla these glands project backward and downward. The enlargement of the ends and constriction of the stalks is more pronounced in this papilla than in those further forward.

The glands of a lateral papilla of an 11.5 cm. fetus are more numerous in connection with the lateral side than with the lower margin of the circumscribing epithelium, whereas in a 12.5 cm. specimen glands extend downward and backward from the lower border alone. A wax reconstruction of a right anterior papilla shows the glands to be more numerous and longer than in papillae located further caudalward. In one instance a gland is divided near its origin, one of the branches being subdivided.

In several instances in a 15 cm. fetus the enlarged oval ends of glands have divided into stalks and end pieces. Some of the stalks are hollow at their origins from the trench which is now present. The trench surrounding the surface of the papilla is well defined although the shape of the papilla can be readily recognized in younger stages by a slight depression.

A model of a papilla made from a 19 cm. fetus shows the trench well developed and gland ducts extending from its lower rim and lateral walls. One exception was noted, namely a gland duct arising from the inner wall of the trench. Some glands are very short, appearing either as knob-like outpouchings or possessing a short, well developed duct and an expanded but still solid end. The ends in some glands are beginning to show superficial subdivisions separated by grooves, indicating acini. Two or three very long ducts extend downward and outward on the lateral side of the papilla at different levels that give off short tubules which terminate in acinar outgrowths, some hollow, others solid. Some ducts divide immediately into two to four branches. No anastomosing ducts were found. Older specimens (23 cm.) showed ducts spreading laterally, others extending deeply into the muscular tissue before breaking up into glandular tissue.

*8. *The weights of the organs in relation to type, race, sex, stature and age.*

(From 115 post mortem examinations at the Charity Hospital, New Orleans, La. Preliminary report.) ROBERT BENNETT BEAN, Anatomical Department, University of Virginia.

The types may be classified in three groups, the hyper-ontomorph at one extreme, the meso-ontomorph at the other, and an intermediate in between. The organs are invariably smaller in the hyper-ontomorph than in the meso-ontomorph, and this difference may be expressed by a number that represents the relative number of large and small organs in each group. This number is a factor that represents the ratio of difference between the hyper-ontomorph and meso-ontomorph in relation to the organ.

TABLE 1
The size of the organs in relation to type

LIVER	KIDNEYS	BRAIN	SPLEEN	HEART	APPENDIX	PANCREAS
6.14	4.10	4.50	4.70	19.00	3.38	4.50

There is greater difference in the heart of the hyper-ontomorph and the meso-ontomorph, and less difference in the appendix. The difference is about the same for the kidneys, brain, spleen and pancreas, but greater than these for the liver.

The differences due to race are determined for the white male and the negro male only. These differences are less than the differences due to type, except in the pancreas.

TABLE 2
The size of the organs in relation to race

LIVER	KIDNEYS	BRAIN	SPLEEN	HEART	APPENDIX	PANCREAS
1.00	0.12	2.33	2.00	0.40	0.50	4.50

The organs are larger in the white male than in the negro male. The numbers given in this and all the tables are obtained by the same method, and indicate relative differences in size. The number is a rough ratio of difference, and any two may be compared with each other in any of the tables.

The differences due to sex are determined for the negro male and the negro female only. These differences are less than those due to type except for the kidneys.

TABLE 3
The size of the organs in relation to sex

LIVER	KIDNEYS	BRAIN	SPLEEN	HEART	APPENDIX	PANCREAS
2.86	3.40	0.60	2.00	14.40	1.30	1.50

The organs are larger in the negro male than in the negro female. The difference is greatest in the heart, and least in the brain.

The differences due to age are less than the differences due to type except in the spleen.

TABLE 4
The size of the organs in relation to age

LIVER	KIDNEYS	BRAIN	HEART	APPENDIX	PANCREAS	SPLEEN
3.50	0.000	1.40	5.15	0.20	0.50	7.75

The greatest difference due to age is in the spleen, and the least in the kidneys. The heart and liver also show differences due to age. More small than large livers, spleens and appendices are found in the old than in the young, and more large than small brains, hearts and pancreases are found in the old than in the young. The brain and heart continue to grow in size with increasing age, whereas the liver and spleen atrophy.

The differences due to stature are considerable, especially for the brain and appendix, in which the differences are greater for stature than for type. The differences in the heart due to stature are unexpectedly small.

TABLE 5
The size of the organs in relation to stature

LIVER	KIDNEYS	BRAIN	SPLEEN	HEART	APPENDIX	PANCREAS
4.85	3.17	13.68	4.87	1.00	6.27	2.40

After all, however, the test of the value of these differences lies in their application to the individual. Is it possible to approximate the size of any organ in the living, if one has the type, race, sex, stature and age of the individual? Let us try a few cases at random. Here is a male negro hyper-ontomorph, aged 52, stature 165 cm. Is the liver large or small? The liver of the negro male should not be so large as that of the white male nor so small as that of the negro female, therefore it should be intermediate in size by race and sex. The age and stature are both intermediate, therefore the type is the most distinctive characteristic. This individual is a hyper-ontomorph and the liver of the hyper-ontomorph is small, therefore the liver of this individual should be small. It weighs 1150 grams which is 350 grams less than the weight of an average or intermediate liver.

Here is a negro male meso-ontomorph, aged 31, stature 169. Is the liver large or small? The race and sex may be disregarded, as before. The stature is only a little above the intermediate, therefore it may be disregarded. The age and the type both indicate a large liver, and the actual weight is 1580 grams.

Here is a white male hyper-ontomorph, aged 50, stature 157. Here the liver should be small but it weighs 2270 grams. The pathological record reads, "amebic abscess of liver," "syphilis of liver." Either one would make the liver weigh more than normal.

Here is a white male meso-ontomorph, aged 56, stature 170. The liver ought to be large and it weighs 2020 grams.

A negro female hyper-ontomorph, aged 36, stature 150, has a liver that weighs 1480 grams which is a little larger than expected.

A negro female meso-ontomorph, aged 18, stature 173, has a liver that weighs 1750 grams, which is about what to expect.

I have gone over the records of the 115 post mortems in this way and in only a few cases was there no explanation of the weight of the organ. If all the factors in each case could be known the weight of each organ might be determined with a fair degree of accuracy during the life of the individual, and the most important factors for such determination are given above.

9. *A case of a persistent vitelline vessel in a human adult.* ALEXANDER S. BEGG, Harvard Medical School.

Attention has repeatedly been called to the persistence of the vitelline vessels as slender cords in certain mammals, particularly in Carnivora, but strange as it appears, reports of cases of such persistence in the human adult are very few. In the time at my disposal, I have found reference to but two cases comparable with the present one. Of these, the first was that described by Spangenberg in 1819, and the second by Hyrtl in 1859. Spangenberg describes his case as showing a vein, while Hyrtl's case, in a child, showed an artery and an accompanying vein. The above papers, together with those of Meckel and Fitz, that of Broman and the more recent exhaustive work on "The umbilicus and its diseases" by Cullen, have been consulted.

The present case was found in an adult female subject in the dissecting rooms of the Harvard Medical School and was kindly given me by Professor Warren for further study. Upon opening the abdomen a strand was noted which ran free from the inner side of the ventral body wall, 5 cm. below and slightly to the right of the navel, down into the pelvis and up amongst the coils of the intestine. The strand was 30 cm. in length and throughout the greater part of its extent was only of the thickness of a coarse thread. The inner end of the strand was attached to the ventral surface of the mesentery at the level of the sacral promontory. Upon taking that portion of the ileum which is 60 cm. from the colon and drawing it forward, the free strand was seen to enter the mesentery midway between the intestinal tube and the dorsal attachment of the mesentery at the level of the promontory. From the mesentery the strand ran downwards crossing in front of the ileum and to the left of its terminal portion, and then turned upwards to join the ventral body wall as stated above. The cord was visible beneath the parietal peritoneum and was seen to terminate in relation to the obliterated right umbilical artery, near the umbilical ring. At

both mesenteric and umbilical extremities the strand became decidedly thickened. Thus if the slender middle portion had become obliterated, there would have remained an appendix meso-ilei, 3 cm. long, and an appendix umbilicalis of 12 cm. (using the terminology proposed by Broman).

The intestine showed no diverticulum, but the strand was directed towards a point on the ileum approximately 500 cm. from the pylorus and 60 cm. from the colon, a region where the remains of the vitelline duct might be expected. There was no trace of an appendix mesoduodeni (Broman) to indicate the former position of the vitelline vein. In fact, no other abnormalities were observed except certain pathological adhesions and the fissures on the under side of the liver indicated that the umbilical veins had been normal.

Upon injection of the superior mesenteric vessels a small branch of the artery was found to enter the proximal end of the strand, but the mesenteric vein showed no corresponding branch. An attempt at injection of the distal end of the strand, through the epigastric artery, was unsuccessful. In Hyrtl's case there was an anastomosis between the persistent vitelline artery and the deep epigastric artery, but such a connection could not be demonstrated in the present case.

An examination of the models made by Dr. Papez seems to show that the strand in the specimen under discussion represents an uncomplicated persistence of the sheath of the vitelline artery, together with a portion of the vessel itself. In Hyrtl's case the artery in a corresponding strand was accompanied by a branch of the superior mesenteric vein, which would seem to be a new formation rather than a persistent vitelline vein. In Spangenberg's case a strand apparently in the same situation is said to have contained a vein only, but this does not accord with normal embryological conditions. The specimen here described is therefore particularly satisfactory since it can be fully interpreted embryologically.

10. *Vestigial gill filaments in chick embryos.* (Lantern.) EDWARD A. BOYDEN, Harvard Medical School.

Since the researches of von Baer, the branchial region in the Amniota has interested biologists as supplying the most conspicuous evidence that the higher vertebrates recapitulate, in modified form, stages in the life-history of their ancestors. Apparently no record has yet been made of structures on the branchial arches of higher vertebrates which could in any way be interpreted as functional or rudimentary gill filaments. While studying the anatomy of the 5-day chick my attention was attracted to ectodermal proliferations, protruding from behind the hyoid arch, which seemed to be involved in the obliteration of the cervical sinus. To Prof. F. T. Lewis I am indebted for the suggestion that these projecting cell clusters might be brought into line with the gill filaments of amphibians and fishes. Subsequent study of older and younger chick embryos, together with the finding of similar structures in turtle embryos, seems to warrant a presentation of this material from a phylogenetic standpoint.

The life history of these filaments, covering a period from the fourth to the eighth day, embraces nearly one fifth of the total period of incubation. Throughout this time the epithelium of the filaments themselves as well as the branchial epithelium which gives rise to them is characterized by the presence of what appear to be degeneration vesicles. These accompany, and thus may be said to register, an activity of the epithelium of which the filaments seem to be the fruition. They first appear, as early as the 76-hour stage, in the posterior walls of the first three pharyngeal pouches at a time when the first three gill clefts have broken through and the fourth pouch touches the ectoderm. When complete, each vesicle is a clear, spherical cyst embedded within the epithelium, containing pycnotic nuclei and cellular fragments. Favorable sections indicate that these cysts result from the nearly simultaneous disintegration of several adjacent epithelial cells.

Following their first appearance, scattered vesicles may arise in the walls of all the pharyngeal pouches and in the ectoderm between the clefts on the outside. About the end of the fourth day they are most numerous in the ectoderm between the second and third clefts and are sufficiently abundant to give it a punctate appearance. Eventually some of them are crowded downward into the underlying tissues. A second, less conspicuous concentration area occurs in the ectoderm between the third and fourth clefts. It is of interest to note that it is the ectoderm of these two arches in frogs and toads which forms the first external gills. Again, recalling the fact that vesicles in the chick first appear in the entoderm, it is well known that a proliferation of entodermal cells in the anura precedes the formation of the gills, but instead of producing vesicles as in the chick, tends to spread out beneath the ectoderm as a secondary layer.

At the beginning of the fifth day the ectoderm covering the third arch, where the vesicles are most abundant, has become considerably thickened and is beginning to produce filaments. As viewed from the outside this arch is wedge-shaped and its downward directed point is the first part to develop filaments. Later the upper portion will also give rise to tufts of cells. In a 12-mm. embryo slightly older than the last, the middle of this wedge actually forms an outpocketing which contains a mesodermal core, thus almost reproducing the early formation of external gills in the Amphibia. In this same embryo the ectoderm of the fourth arch forms an evagination, which however is solid and much smaller, and tends to fuse with that from the third arch.

During the remainder of the fifth day the hyoid arches meet in the mid-ventral line and begin to grow backward over the posterior arches, as in the Anura, in the form of a gill-cover or operculum. It carries with it, on its under surface, the tufted epithelium of the third arch, and from now on the filaments of this region of fusion will appear to come from under the surface of the operculum. The filaments attached to the operculum will always be the largest of the series and persist the longest. Differentiation lateral to this point on each side continues

slowly, until, toward the end of the sixth day, there is a transverse line of filaments on either side extending in a dorsal direction part way across the neck. In the next few hours this may be supplemented by a ventral extension of filaments along the under surface of the operculum until in some specimens they nearly reach the mid-ventral line. It is about this time that the larger filaments regularly show branching. This period of maximum extent in the first quarter of the seventh day is followed by a rather rapid decline, until, at the end of this day, all trace of operculum and filaments has disappeared. Coincident with this decline is a curious median proliferation of epitrachial cells in the cardiac region. This is being studied further, but apparently has no connection with the subject under discussion.

By way of a summary the life history of these vestigial structures may be divided into five stages: 1, the *appearance of degeneration vesicles* in the branchial epithelium; 2, the *concentration of these* in the ectoderm covering the third arch and, to a lesser extent, that covering the fourth; 3, the *thickening of the ectoderm* of these two vesiculated areas into tufted epithelial mounds, and, in the case of the first, an apparent *evagination* of the ectoderm with a mesodermal core; 4, a gradual *differentiation of these areas* (now crowded into one and fused with the sides of the backward growing operculum) *into a transverse line of filaments* on each side of the neck; 5, a rather *rapid reduction* of this line and the eventual suppression of both filaments and operculum.

In conclusion, attention should be called to the recent experiments of Ekman ('13) which have a bearing on this problem. He was able to show by transplantation methods that the ectoderm of the branchial region of frogs and toads has a certain specificity for building gill filaments not possessed by the remaining ectoderm of the embryo; that a polarity of this ectoderm could be demonstrated; and that even when the entoderm and mesoderm underlying the future gill region were removed the ectoderm alone could produce abortive filaments. It is the ectoderm of this same region in reptiles and birds which produces rudimentary filaments and they bear a striking resemblance to some of the abortive structures produced experimentally in Amphibia by Ekman. In the case of higher vertebrates the process never passes beyond the initial stages, as evidenced by the early appearance of degeneration vesicles and the failure of blood vessels to participate in gill formation.

11. *Development of the preoptic part of the forebrain of Amia calva.* CHAS. BROOKOVER, University of Arkansas, Little Rock.

In connection with a study of the adult brain of *Amia* it was thought best to model some stages of the development of the forebrain in order, if possible, to bring it and the closely related divergent forebrain of the teleosts into closer harmony with the ancestral vertebrate brain. The early stages, as expected, show a simpler and more diagrammatic condition than the adult.

The earliest stage modeled is 5 mm. long, taken at a time just before hatching. The embryological condition of an epithelial tube exists. It is bent so as to throw the pineal anlage to the anterior and is but slightly thickened in two places on either side of its basal part. The anterior swelling into the neural canal is beneath the fibrous connection with olfactory placode and goes later into the formation of the olfactory bulbs. The posterior one is the derivative from which comes the remainder of the preoptic forebrain. A fibrillar zone at the periphery permits mapping out the anterior commissure, the olfactory tracts, the optic tracts and perhaps the habenular tracts (fimbria). On the ventricular surface the slightly thinner pallial portion is marked by upper and lower parallel lines running just dorsad of the lateral olfactory tract. The ventricular sulcus bends caudoventrad to flatten out and be lost in the diverticulum of the optic stalks.

Larval stages of 8 and 10 mm. total length show a previous rapid development of the olfactory centers. The thickness of the olfactory bulbs shows in an external swelling and an internal rhinocoele has been developed. Neuroblasts for the formation of mitral cells have migrated toward the periphery. Posteriorly in the olfactory lobes the cells for the formation of the lateral olfactory area have proliferated. This makes a dorsal swelling. Ventrally the cells keep for a longer time their original position and largely epithelial condition along the walls of the common forebrain ventricle. This ventricular lumen extends forward somewhat beneath the olfactory bulbs but not so far as in the earlier stages where it produces what has been called an unpaired olfactory placode.

The above mentioned cells along the ventral portion of the forebrain ventricle are continuous without line of demarcation into the rhinocoeles and here the cells of the nucleus olfactorius anterior of authors are proliferated. In the forebrain this undifferentiated epithelial zone extends posteriorly over the commissure into the thalamus. The medial olfactory tract somewhat belated in its development comes into relation with the anterior part which develops into the nucleus of the precommissural body. This is not exclusively olfactory in function. The nervous terminalis passes through it and the ascending fibers to the olfactory bulbs originate from it in some forms. Golgi preparations of larval *Amia* at this age show ascending as well as descending fiber connections between this region and the thalamus. Fishes of 22 mm. and of 47 mm. length have a ventricular sulcus extending from the rhinocoele dorsocaudad to the neighborhood of the velum transversum. This sulcus (*limitans telencephali*) becomes less evident with age and the pressure of opposite sides of the hemisphere on each other. This pressure is due to the large lateral and medial olfactory areas lying dorsal and lateral to the sulcus, as well as to the striatum (*paleostriatum*) lying laterally.

At the anterior end of the sulcus *limitans* the cells of the islands of Calleja originate and migrate laterally into position between the two olfactory tracts. Adjacent and continuous with these within the

olfactory bulbs the nucleus olfactorius anterior proliferate. The olfactory bulbs remain in embryonic (primitive) proximity to the fore-brain.

In its middle portion the cells of the region just ventral to the sulcus limitans give rise to the paleostriatum. At its caudal end near the velum transversum three nuclei connected to the habenula originate from the ventral edge of the sulcus, viz., the nucleus theniae carried laterally in the eversion of the hemispheres, the nucleus lateralis commissuralis (Sheldon on the carp), and a thalamic nucleus remaining in its primitive position near the ventricle. The second may be connected only with the ventral lobe of the habenula.

The forebrain of *Amia* is primitive in the proximal position of its olfactory bulbs and the location of its nonolfactory centers near the ventricle but specialized in its hypertrophied olfactory nuclei with strong habenular connections.

*12. *Histological differences between certain muscles of the cat as related to physiological and chemical differences.* (Lantern slides.) H.

HAYS BULLARD, Pathological Laboratory, Johns Hopkins University.

The object of this paper is to call attention to some morphological differences between certain muscles of the cat which are believed to correspond to a number of the physiological and chemical differences in the same muscles set forth by Lee, Guenther, Meleney, Scott and Colvin in a series of interesting papers appearing in *The American Journal of Physiology*, May, 1906. In one of these papers (Lee, Guenther and Meleney) it is mentioned that the authors could find in the literature no comparative histological study of the muscles under discussion, namely, diaphragm, extensor longus digitorum, sartorius, and soleus. From their own histological observations they were not able to point out any striking differences between the given muscles.

In the present experiments these four muscles from twelve cats (young and adult) have so far been examined. The tissue was fixed in 20 per cent formalin and frozen sections were stained with Sudan III by Herxheimer's method. This method is well adapted to the demonstration of the dual structure of muscle as seen in the so-called light and dark or cloudy fibers. The occurrence of an intermixture in varying proportions of these two types of fibers in the skeletal muscles of many animals, including probably all vertebrates, has been known for a long time (Knoll). As is also well known light fibers usually have a few small fatty droplets in their cytoplasm. Dark fibers have many larger fatty droplets. Intermediate fibers also occur which are likewise fatty and probably belong to the dark type.

The four different muscles of the cat may be characterized, briefly, as follows: *Diaphragm*. Light and dark fibers in about equal number, small number of intermediate fibers. Average diameter of fibers less than in other three muscles. *Extensor digitorum*. Light fibers form more than half the total number, dark and intermediate in about

equal number. Average diameter of fibers greater than in diaphragm and less than in soleus. *Sartorius*. Dark fibers predominate, intermediate and light fibers in about equal number. Average diameter of fibers greater than in diaphragm and less than in soleus. *Soleus*. Intermediate fibers predominate, dark fibers in considerable number, light fibers either absent or of very infrequent occurrence. Average diameter of fibers greater than in the other three muscles.

In all four muscles light fibers are of greater diameter than dark. When these four muscles, only, are considered the microscopical picture of each is usually sufficiently characteristic to permit of its identification. From the predominance of intermediate and dark fibers it is always possible to identify the soleus, in transverse section, from a single low power field. The predominance of light fibers serves for the easy identification of the extensor digitorum. The diaphragm and sartorius are frequently identified only after several fields are examined and, exceptionally, one of these muscles is mistaken for the other.

The extensor digitorum and sartorius are macroscopically pale muscles. The soleus is a red muscle and the diaphragm is intermediate in color. It is not true, however, that the dark fibers of the soleus are the cause of the dark or red color of that muscle. Dark fibers have their characteristic appearance only when seen by transmitted light under the microscope. By reflected light they appear light. It follows that dark fibers tend to make the muscle appear pale and the dark red color of the soleus is not due to its dark fibers. Also it is known that many pale muscles are composed largely of fibers that are microscopically dark.

The extensor digitorum, according to Lee, is characterized by great irritability, a short latent period and quick contraction and relaxation while the soleus is less irritable and after a longer latent period it contracts and relaxes slowly. As mentioned above the histological picture shows a great predominance of light fibers in the quick muscle (extensor) and a predominance of intermediate and dark fibers, with almost total absence of light fibers, in the slow muscle (soleus). The diaphragm and sartorius occupy an intermediate position both in these physiological properties and in the relative number of light and dark fibers. It is not probable that these facts are unrelated.

In the present experiments the histological findings in respect to the relative fat content of the four muscles agree with the chemical analyses of Lee. The extensor digitorum, both chemically and microscopically, shows considerably less fat than do the other three muscles. Moreover the quantity of fat microscopically demonstrable in each of the muscles is so great that it would appear to account for the entire quantity shown by chemical analysis. This is not in accordance with the accepted view that much of the fat in the tissues is chemically combined and not capable of microscopical demonstration.

In fasting animals, Lee has shown that the total working power of the diaphragm is reduced by 44 per cent while the working power of

the extensor is reduced by 16 per cent. It is known that fat gradually disappears from the fatty dark muscle fibers of fasting animals (Knoll, Bell, Bullard). As we have seen the diaphragm of the cat contains many more fatty fibers than does the extensor. When the fat is removed by starvation one would expect the diaphragm to undergo a greater proportional reduction of working power.

It is scarcely necessary to mention that the microscopical differences between the four muscles under discussion are not here considered exhaustively. Interesting details respecting nuclear and myofibril characters, occurrence and distribution of mitochondria and glycogen, blood and nerve supply, and connective tissue distribution, all remain to be investigated. A study of the length of the muscle fibers by the methods recently described by Huber might also prove of considerable interest.

There is good reason to believe that the striking physiological and chemical differences which Lee and his collaborators have demonstrated in these four muscles are paralleled by no less remarkable morphological differences.

13. *Some factors regulating growth.* MONTROSE T. BURROWS, Department of Pathology, Johns Hopkins University.

The problems under consideration in this paper are (1) the nature of the immediate conditions which lead to the failure of scar formation in many wounds or following many extensive inflammatory processes and (2) the general nature of the conditions which inhibit or allow the growth of connective tissue. It is well known that the most extensive inflammations of epithelial surfaces, as pneumonia, are most often followed by complete healing, while inflammations of the deeper connective areas are most frequently followed by the formation of a scar. In cancerous processes the connective tissue cells grow wildly at the expense of other parts.

Hertzler, a few years ago, came to the conclusion that the fibrinous exudates which forms in a wound, is the direct stimulus for the growth of the connective tissue cells. He noted that skin grafts take only when they become imbedded in a layer of coagulable exudate. He also thought that the fibrin fibrils were transformed directly into the extracellular connective tissue fibrils. He had come to this last conclusion by means of a careful chronological study of intestinal adhesions and wounds induced by mechanical means in young rabbits of the same litter. He noted that previous to healing the fibrin is laid down in the form of fibrils. Connective tissue cells migrate among these fibrils. At a later period he found the fibrous tissue fibrils to occupy the same position and have the same arrangement as the fibrin fibrils. The wounds and adhesions had been removed at regular intervals, sectioned and stained. He was unable to see any evidence of the disappearance of fibrin, and the laying down of the connective tissue fibrils. Similar experiments were also made with wounds. In the summer of 1908, the author had the opportunity to study these experiments with Dr. Hertzler.

In the early studies of tissue culture made at Cornell University Medical College, it was noted that the fibrin fibrils in many of the cultures, after a considerable growth of connective tissue cells, stain the characteristic pink color, of white fibrous tissue with Von Gieson stain. These observations were communicated to Dr. Hertzler, who reported them with his own studies ('13). Recently, Baitsell ('16), in Harrison's Laboratory, has made similar observations in tissue cultures. He did not observe the characteristic color reaction with Van Gieson stain.

Whether or not these pink staining fibrils that had been observed in the tissue culture could be considered as true fibrous tissue, or merely structures simulating these fibers in their ability to absorb dyes, was a problem of interest. One of the objections to accepting them was the inconstancy of the appearance of pink staining fibrils in many of the cultures.

In later studies of the growth of tissue in vitro several facts have been found, however, which would tend to substantiate this particular view. The first is that clot contraction or the formation of fibrin fibrils in the cultures of chicken tissue in plasma occurs only in the presence of connective tissue cells. Chicken plasma, when carefully prepared, free from previous tissue contamination, clots with the addition of a fragment of tissue to form a practically structureless jelly-like mass which has the same volume as the original fluid plasma. Any type of tissue conditions this primary clotting. In the presence of living connective tissue cells the clots later undergo contraction, while in the presence of epithelium they may undergo contraction but later liquefaction. Leucocytic or lymphocytic cells cause only slight liquefaction of these clots and very little, if any, contraction.

The second fact is that this contraction takes place only in the presence of living connective tissue cells and then only after a considerable latent period. It fails entirely when the oxygen is replaced by nitrogen or hydrogen. It also fails when the tissue fragments have been heated for five minutes at 60°C. *In other words, it is evident that clot contraction is a phenomenon instituted by conditions quite different from those of primary clotting and it is a phenomenon which is brought about by the action of the products of metabolism of the connective tissue cells.*

At another time the author studied more carefully the properties of the connective tissue cells. He has found that the cells of higher animals are not highly organized, but fluid-like systems. Their various manifestations of life such as movement, growth, etc., are differential surface tension phenomenon under the control of a specifically organized environment. The food materials or energy producing substances in the cultures are not derived from the medium, but from the cells within the fragment. The growth that one observes in the cultures is none other than a simple transfer of materials from the cells of the center of the fragment or in a less favorable environment to those on the periphery or those which have been carried out into the medium through the interchange of substances between the fragment and the

medium. This was shown by the fact that the cells can be grown in simple salt solution and, in the plasma cultures, growth cease after a few transplants, the sum of the total growth being less than the original mass - or it represents what one might assume the original mass minus the energy of transfer. The cells that tend to break down in the fragment and lead to the greater growth of the connective tissue cells are not the connective tissue cells but the epithelium, muscle cells, etc.

Again it was noticed that this growth takes place only in the presence of oxygen. It commences after a given latent period in the case of connective tissue, subsequent to the contraction of the clot). The cells grow actively for a time gradually to come to rest. This reaction is one which apparently commences subsequently to the slow diffusion of substances between the fragment and the medium and proceeds until an equilibrium is established. In other words, it follows the curve of reaction of a heterogeneous physical chemical system. The cells at the end of this reaction do not undergo, at least for a considerable time, any further change. They show no immediate disintegration. That this cessation of growth is not due to the exhaustion of oxygen or food materials is further shown by the failure of any change in the cells following the introduction of fresh air into the culture chamber, and by the fact that activity is again seen when the cells are transplanted to a new culture medium. On the other hand, that it is due to the accumulation of waste products is shown by the fact that the cells which tend to survive for the longer periods of time are invariably those cells which had grown out into the clot and have become completely surrounded by the contracted fibrin. It was of general interest in making these observations to note that this equilibrium which had been established in the tissue culture did not alone concern the cells which had migrated out and grown in the culture medium, but likewise those which remained within the fragment. With the cessation of growth of cells in the outer medium, disintegration with the liberation of energy-producing substances in the fragment also ceases. This is especially true when the fragments have been placed in thick layers of plasma, so that they have become likewise completely surrounded by contracted clots. Many such cultures were kept for as long as six months at incubator temperature and in an ample supply of oxygen before any disintegration became apparent. Several were transplanted at this time and an active growth of cells was observed. The growth of the connective tissue cell is apparently a tissue and not a cellular reaction. The failure of the connective tissue cells to dissolve the fibrin and their ability to transform it into fibrils, leads to the belief that these fibrin fibrils form the superstructure upon which, or out of which, the connective tissue fibrils are built. We observed pink staining fibrils only in cultures of skin of foetal chickens. Whether the formation of the connective tissue fibril is a body, rather than a connective tissue, cell reaction is a question for solution. It is well known that the growth of cells in the animal organism is not determined alone by food and oxygen but by other unknown

conditions. The question naturally arises, have these unknown conditions been found. Are the actual waste products of metabolism of these cells, substances which are insoluble in body fluids and is the cessation of growth of a part, the result of the accumulation of these substances? One may assume that the fibrin fibrils are formed by the action of the products of metabolism of connective tissue cells on the coagulable exudate, and that the cessation of growth in the wound is due to the accumulation of these substances in and about the growing cells. From these observations, one might readily reduce stimulation as any condition which would lead to the reduction of concentration in these substances. The stimulating action of fibrin is due to its ability to absorb these substances. To prove this more completely the author studied rhythmical activity in heart muscle cells as well as the growth of these and of connective tissue cells in plasma cultures so arranged that the media could be continuously washed with a stream of serum. The rhythm of heart muscle fragment in simple hanging drop cultures is invariably intermittent. In the body it is a form of activity which continues throughout the life of the individual. In the cultures, where the media was continuously washed by a stream of serum, the rhythm was not only greatly prolonged up to the time of complete exhaustion of the cells but it continued regular, while the growth of the cells was not changed but similar to that seen in the simple hanging drop cultures. In a former paper before this Society, the author presented facts to show that the contracting, embryonic heart-muscle cell has an organization which one might readily assume capable of splitting these insoluble waste products into simpler substances and of transforming the energy liberated with their formation into work of contraction.

Certain rapidly growing tissues, such as embryonal and rapidly growing tumors grow readily in liquid medium. This growth takes place only near the surface of the liquid. Cells suspended in liquid invariably round off and show no activity. The cells do not grow out into the liquid. It is of interest to note that adult tissues do not, however, grow readily in liquid medium while, on the other hand, they grow actively in plasma.

It was in the light of these facts and the more careful study of the properties of epithelial as well as leucocytic and lymphocytic cells that the general deductions as to the cause of the failure of scar formation in superficial inflammations of the epithelial surfaces was derived. Epithelial cells invariably bring about a rapid dissolution of the fibrine lots. When occurring in considerable numbers in a fragment of tissue, they invariably prevent entirely a growth of the connective tissue cells. That the appearance of organization in the pneumonic lung probably indicates a complete destruction of the epithelium rather than the lack of leucocytes in the exudate was further suggested by the fact that the leucocytic infiltration in deep seated inflammation is frequently as great as in the superficial ones. It is true that the leucocytes of man are richer in ferments than those of

lower animals; the failure to observe any extensive liquefaction about the leucocytes of chickens would not indicate that this did not occur in human beings. On the other hand, it has been found that fragments of human connective tissue containing leucocytes grow readily in plasma clots when they are removed after twenty-four hours from the first culture to a drop of fresh plasma. This is not true of epithelium. The cells continue to liquefy the plasma, even after many transplants, or until they are dead.

These observations are reported not only for the general bearing that they have on the nature of stimulation and the significance of extracellular substances in their relation to life processes, but also for their immediate significance for the better understanding of the actual conditions which regulate the growth of body cells. If these experiments are substantiated, showing as it is believed they do, that growth is inhibited by the accumulation of insoluble waste products and permitted to proceed only by their removal, then the problem of the growth of the cell is brought into the domain of chemistry. Thus problems, such as those that confront us in cancer, are narrowed.

**14. Preliminary report on the normal unequal growth and degeneration in the early ossification centers in the diaphyses of femora of the pig.*
EBEN CAREY, (introduced by F. W. Heagey), Department of Anatomy, Creighton Medical College.

The normal unequal growth of the subperiosteal osseous tissue, and of the degeneration of the hyaline cartilage cells and matrix in the early advancing diaphyseal center of ossification, as in the femur of the pig, have not been made an intensive problem by investigators. As a consequence descriptions of the early development of the diaphyses of long bones are so worded as to avoid clear statements on certain fundamental points. This paper will be limited to the time when the primary subperiosteal osseous lamina, or as it here will be designated, the lamina prima, is fairly well differentiated.

1. *Material and methods.* The pig was selected for this problem on account of the abundance of material procurable by the proximity of the laboratory to the South Omaha abattoirs. The femur was chosen as a prototype of long bones with two epiphyses; it was also chosen because the ventral, lateral and mesial walls of the diaphysis of the adult femoral bone primarily present a laminar type of formation and not the characteristic Haversian system type as found in the human femur (Foote '11-'16). This report is a part of a more extended study considering the complete embryology of the pig's femur.

The embryos were hardened by both the alcoholic and Zenker's fixation methods. In the former no decalcification takes place and it was used to ascertain the time when the precipitation of calcium salts precludes sectioning. By the latter method the earliest center of ossification is easily decalcified without subsequent subsection to a stronger decalcifying agent. The center of ossification of older bones,

after Zenker's fixation, are prepared for sectioning by von (Ebner's) decalcifying fluid which was used with excellent results. With the latter reagent less distortion, due to the swelling of the collagenous fibers, took place.

Both cross and longitudinal serial sections were prepared of the femora. Some of the sections were stained with Delafield's haematoxylin counterstained with alcoholic eosin, others were well defined with Mallory's connective tissue stain.

2. Inception of period of ossification. In embryos with a crown-rump measurement of between 20 to 22 mm. the cartilaginous femur is approximately 2 mm. in length. It is well outlined, shows certain adult characteristics, and cavity formation has just begun in the tissue lying between the cartilaginous floor of the acetabulum and the head of the femur. In the first step of the formation of the cavity at the knee joint, there is a condensation of the capsular tissue immediately bordering upon the joint and of the perichondral tissue which at this stage covers the cartilages on their articular surfaces as well as elsewhere. The cartilage of the shaft is of the cellular variety and presents an epithelioid appearance. At either extremity the pre-cartilaginous cells predominate.

It is at this stage that the first changes of osteogenesis are noticed. There is an unequal growth of the osteogenetic cells of the Cambium layer of the periosteum, which encircles the shaft. More cells are proliferated and the constriction is slightly deeper on the ventro-mesial aspect than elsewhere. The cellular cartilage begins to appear slightly atrophied and vesiculated, as evidenced respectively, by the more shrunken granular cells and by a few hollow vesicles, immediately underlying the zone of more vigorous proliferation of osteogenetic cells.

The osteoblasts ultimately form the lamina prima or the first embryonic osseous layer encircling the degenerating cartilaginous shaft. This process also begins on the ventro-mesial aspect. The unequal growth of this lamina prima was followed through the 3 mm., 3.5 mm., 4 mm., 4.5 mm. and 5 mm., lengths of the femur, in embryos with a respective crown-rump measurement of 28 mm., 30 mm., 34 mm., 38 mm., 41 mm. These measurements are the mean computed from five series of 8 to 10 embryos in each series.

3. Conclusions. *a.* On the ventro-mesial aspect of the central osteogenetic cellular constriction there is a more active proliferation of osteoblastic cells than elsewhere. Immediately underlying this zone the processes of atrophy and vesiculation in the cellular cartilage are begun.

b. The lamina prima first differentiates in a 3 mm. femur on the ventro-mesial aspect, quickly encircling the center of the degenerating cartilaginous shaft as evidenced by the growth in a 3.5 mm. length of the femur

c. There is a more rapid advance of the developing lamina prima towards the proximal epiphyseal line at the head end of the femur than towards the distal epiphyseal line at the condylar extremity of the shaft.

d. Calcification and vesiculation of the cartilaginous matrix and cells, respectively, also advances at a more rapid rate towards the proximal head end of the femur keeping slightly in advance to the rapidly progressing lamina prima.

e. The lamina prima on the ventro-mesial aspect reaches the proximal epiphyseal line before that on the dorso-lateral aspect. This is the case in a 5 mm. femur and the lamina prima is also considerably thicker on the former than on the latter aspect.

f. A lamina secunda is differentiating, in the 5 mm. length of femur, at the central primary constriction peripherad to the lamina prima.

g. A significant fact is that the nutrient artery enters the adult bone in the upper one-third of the diaphysis on its ventral aspect and is directed distally towards the condyles.

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*15. *Microdissection studies.* Cell and nuclear division. (Lantern).

ROBERT CHAMBERS, Jr., Cornell Medical College, New York City.

Cortical changes in the egg cell on the approach of cell division can be demonstrated with the needle. In polar body formation the change appears to be due to the presence of the nucleus for, on removal of the nucleus to another region of the cell, a corresponding change in the cortex occurs. Preparatory to division the bulk of the cytoplasm undergoes temporary gelation and constriction takes place in the region where liquefaction sets in.

The resting nucleus is an optically homogeneous body in which a granular network is often produced on the slightest injury. It is fluid in consistency in egg cells and possibly in other cells. Formation of the nuclear spindle was studied in germ cells and in egg cells during polar body formation and segmentation. In no case could spindle fibers be demonstrated. The nuclear substance assumes a spindle shape under the influence of the centrospheres. Liquefaction of the cytoplasm in the equatorial region of the cell is accompanied by a constriction which shapes the nuclear spindle into a dumb bell during the ana- and telo-phases. As the daughter nuclei draw away from one another they are connected by a strand which is of a sufficient consistency to distort the nuclei if caught and pulled by the needle.

The prechromosomal filaments appear in the prophase out of the hyaline nuclear matrix through the precipitation of granules in more or less irregular masses closely investing long slender cylindrical strands of a gel like consistency. Many of the diplotene filaments figured in fixed material may possibly be due to the collapse of these strands into ribbon like structures with an accumulation of the granules along the edges. Shortening of the prechromosomal filaments is ac-

accompanied by a fusion of the granules thus forming the definite hyaline, viscous and comparatively rigid chromosomes. The chromosomes are imbedded in the nuclear spindle mass and undergo movements resembling those in drops which are undergoing changes in surface tension. The constriction of the nuclear spindle at its middle possibly aids the migration of the chromosomes by pushing them to the poles.

Many if not all of the structures which appear during cell and nuclear division may be explained as reactions in a mixture of colloidal materials producing local liquefactions together with precipitations and gelatins which may be made to disappear on changing the reaction.

16. *A study of the reaction of lymphatic endothelium and of leucocytes in the tad-pole's tail toward injected fat.* ELIOT R. CLARK and ELEANOR LINTON CLARK, Department of Anatomy, University of Missouri.

The present investigation is part of a series undertaken in order to study the growth and reactive powers of living tissues and cells, in the tad-pole's tail, by observing their response toward various injected substances. One of the authors has reported the results obtained by injecting small globules of paraffin oil. He showed that these globules were not absorbed and that, aside from a transitory leucocytosis, the various tissues and cells showed no reaction to the injected paraffin oil. In the present study, small amounts of various fatty substances were introduced subcutaneously, with the especial object of studying the reaction of the lymphatic capillaries. The substances injected were olive oil, oleic acid, cream and yolk of egg.

1. *Olive oil.* The oil was injected in the form of single globules, measuring about $50\ \mu$ in diameter. The globules diminished in size during the period of observation but were not completely absorbed after nineteen days. Soon after the injection, clear leucocytes were seen to pass through the walls of nearby blood-vessels and to approach the oil. Here they flattened out and formed a ring of leucocytes around the periphery of the globule. Soon after coming in contact with the oil, they became pigmented and were seen to contain small drops of oil. The lymphatic capillaries were evidently attracted by the presence of the olive oil; they grew toward it, even bending out of their course in some instances. Upon reaching the globule, the lymphatic tip remained in close contact with the rim of pigmented leucocytes for several days. In some cases, the tip of the lymphatic later extended beyond the oil globule. No pigmented leucocytes were seen to enter a lymphatic capillary.

2. *Oleic acid.* Immediately after injection, the oleic acid changed from a clear refractile globule to an opaque granular mass which was brown by transmitted and white by reflected light and resembled closely the sodium soap of oleic acid. The leucocytes responded to this substance more quickly and in larger numbers than in the case of the olive oil. They formed a ring around the injected mass several layers deep and all became deeply pigmented. On the day following the

injection, many small refractile droplets could be seen, scattered through the brown substance. The absorption proceeded more quickly than in the case of the olive oil but was not complete after ten days. The lymphatics responded, as in the case of the olive oil, by growing toward the injected substance. Pigmented leucocytes were observed to move away from the injected mass and to come into close contact with the tip or wall of nearby lymphatic capillaries. After remaining for five or ten minutes in close proximity to the lymphatic, they moved away again and, shortly before or at the time of wandering away, these leucocytes lost their pigment and became clear.

3. *Cream and yolk of egg.* Both of these substances consist mainly of an emulsion of very small droplets. The leucocytes were attracted to the injected cream or yolk in large numbers and actively took up the minute drops of fat. The leucocytes containing fat were then seen to wander off and to come into close relationship with nearby lymphatic capillaries. After remaining close to the lymphatic for a few moments, they became clear again. At the end of twelve hours, most of the injected droplets had been taken up by leucocytes, and after twenty-four hours all had been. On the second day, only a few pigmented leucocytes marked the site of injection. In spite of the rapidity of the absorption in the case of the yolk and cream injections, a definite growth of lymphatic capillaries toward the injected region was observed in some instances:

Conclusions. a. Lymphatics reacted to the injected fat by sending out sprouts which grew toward it.

b. Leucocytes responded quickly to the injected substances, migrated toward them in large numbers and actively engulfed the fat.

c. The fat appeared to be absorbed through the combined efforts of leucocytes and lymphatics.

d. Mesenchyme cells and blood-vessels did not respond to the injected fat.

e. The rapidity of the absorption depended upon the size of the fat droplets: the fine emulsions of cream and yolk were taken up very much more quickly than the single relatively large globules of olive oil and oleic acid.

f. The fat appeared to be changed within the leucocytes and to be absorbed in a soluble form by the lymphatics.

17. *Some points on the urogenital system of myxinoids.* J. L. CONEL (introduced by H. D. Senior), University and Bellevue Hospital Medical College.

A study of the adult structure of the urogenital systems of *Myxine* and *Bdellostoma*. The work was done in the Zoölogical laboratories of the University of Illinois.

A central duct is present in the pronephros of both *Bdellostoma* and *Myxine*, but it is in a state of degeneration in both animals. This degeneration is more advanced in *Myxine* than in *Bdellostoma*, and includes the inner ends of the pronephric tubules and all parts of some of the largest tubules.

The Malpighian body of the pronephros in both *Bdellostoma* and *Myxine* is located at the posterior end of the pronephros, and in appearance and structure closely resembles the Malpighian bodies of the mesonephros. It is formed by the fusion of two or more glomeruli.

The glomerulus of the pronephros in young *Myxinoids* is exposed to the pericardial cavity through a large opening, thus resembling a glomus. This opening becomes constricted to a small tubule in adult animals.

The tubules of the mesonephric Malpighian bodies in *Bdellostoma* are structurally of two types.

Neither *Bdellostoma* nor *Myxine* is a protandric hermaphrodite.

*18. *On the lipoidal nature of structures in the corpus luteum cells of swine.*
GEORGE W. CORNER, Hearst Anatomical Laboratory, University of California.

In a previous paper (*The Corpus Luteum of Pregnancy as it is in Swine*, Carnegie Institution of Washington, Contributed to Embryology, 5) the author called attention to certain structures appearing in the lutein cells of swine after fixation in formol, in the form of small hollow spheres lying in spaces in the outer part of the cytoplasm. They are probably similar to bodies found in the lutein cells of rabbits by Franz Cohn (*Arch. f. Mikr. Anat.*, 62, 1903). In sows they occur only during the first half of pregnancy, and the changes leading to their disappearance from the cells are so characteristic that by examining the corpus luteum it is possible to estimate the stage of the pregnancy with some accuracy.

It was tentatively suggested, in the former paper, that these bodies represent an elaborate modification of the Golgi-Holmgren intracellular canalicular apparatus. However, preparations since made, by Cajal's uranium nitrate technique, show the above described structures and the Golgi net to be present in the same cell and independent of each other. It has been found that they are not present in the fresh tissue, appearing only after several hours in formol, Zenker's fluid, or other aqueous fixatives. They are stained blue by Nile-blue sulphate, pale brick-red by neutral red, give a brown lake with Weigert's hematoxylin, stain positively with Ciaccio's method, and are not anisotropic when seen under the polariscope. They do not stain with Nile-blue after treatment with alcohol of 65 per cent or stronger, and stain but faintly or not at all after a day's immersion in acetone, chloroform, ether, xylol, and benzene. It seems clear, therefore, that the appearance of the spherules is produced by the swelling into rounded masses, in the presence of water, of a lipid, which is present in the luteum cells and which is probably a phosphatid, to judge from its microchemical reactions. Apparently, however, in the process of swelling of the lipid material some proteid is carried with it, and is precipitated in the spherules during fixation, for even after removal of the lipid substance by alcohol, the skeletons of the spheres remain, and may be stained by appropriate dyes, especially Mallory's con-

nective tissue stain. The hollow center in such stained preparations is due to the fact that the spherules usually form about the globules of neutral fat which the lutein cells of early pregnancy contain in great numbers, and the spaces about them are due to shrinkage of the cytoplasm.

The discovery that the appearance of these bodies after fixation is due to an artefact does not detract from their interest, since they enable us to detect the presence of a constituent of the cell which may be of importance in the economy of the corpus luteum, and to follow histologically the changes in amount of this substance.

*19. *Oestrus and ovulation in swine.* GEORGE W. CORNER and A. E. AMSBAUGH, Hearst Anatomical Laboratory, University of California.

In order to obtain a point of departure for studies on the changes of the uterus and ovaries during the reproductive cycle in swine, we have undertaken to determine what relation exists between oestrus and ovulation. Breeders agree that oestrus occurs every eighteen to twenty-one days, usually lasting three days. (One of our animals showed signs of heat on four successive days.) Heat is not terminated by copulation, which may occur repeatedly. We have found that animals killed during this period usually show recently ruptured Graafian follicles, and in such animals we have been able to recover the ova by washing out the Fallopian tubes. Rupture of the follicle is spontaneous, occurring even in the absence of the boar. In one animal in which oestrus was noted sixteen hours before killing, copulation almost certainly having occurred during the interval, rupture of the follicles had not yet taken place, although sections showed an intact follicular wall with a normal ovum in which the first polar body was forming. It is clear, therefore, that ovulation occurs during oestrus, and probably on the first or second day of the period, since we find regularly that ovulation has taken place when sows are killed on the third day of oestrus.

The unfertilized ripe ovum of the sow, as found in the tube, measures 155 to 165 μ in diameter. The zona pellucida is 7 to 8 μ thick, enclosing a yolk heavily laden with fat globules, obscuring the nucleus. The polar bodies are often clearly seen in the fresh ovum. Study of a small series of ova which have been cut into serial sections seems to show no deviation from the stages reported in other mammals; the first polar body is formed within the follicle just before rupture, the second in the tube. Entrance of the spermatozoön and fusion of the pronuclei occurs in the tube.

20. *Potentialities of the lymphoid hemoblasts of the adult spleen.* (With demonstrations.) VERA DANCHAKOFF, College of Physicians and Surgeons, Columbia University.

The adult spleen is a lymphopoietic organ. Under normal conditions numerous small lymphocytes are developed in its follicles. The stem cells of the small lymphocytes are morphologically similar to the stem

cells of other blood cells, either granuloblasts or erythroblasts. The morphological structure of all these stem cells or lymphoid hemoblasts is permanently retained by the organism from the time of the first appearance of blood.

Embryogenetic studies have shown that the various differentiations of the hemoblasts is associated with different environmental conditions. A hemoblast under definite environmental conditions can split off only one kind of blood cells. What is the action exerted by the environmental conditions on the hemoblasts? Do they alter the metabolism of the hemoblast irreversibly, definitively narrowing its potencies to the limits of its prospective value, or do they merely condition its differentiation for the time in which they act, temporarily inhibiting some of its potencies, which may subsequently become active in other environments?

If the normal differentiation into a small lymphocyte is forced upon a hemoblast in the follicle of the spleen by definite environmental conditions, other potentialities should be realized by the same cell under other environmental conditions.

A mesenchymal cell in the allantois responds to a stimulus or to an irritation in a characteristic manner, it readily proliferates; if situated in a well vascularized region, it rounds up, becomes mobile, transforms into a hemoblast; finding itself outside of the vessels it differentiates into a granuloblast and finally into a granular leucocyte. The stem cells in the lymphoid follicle of an adult spleen do not differentiate under normal conditions into granular leucocytes. Would this differentiation take place if the follicle of an adult spleen were transplanted into environmental conditions, favorable to the granuloblastic differentiation? If the potencies of the stem cell in the follicle of an adult spleen, besides its own prospective value, include the prospective values of the stem cells of granuloblastic tissue, then such differentiation of necessity must occur.

Cultures of spleen tissue of different animals were made on the allantois of the chick embryo. The cultures grew well and formed tumors of nearly 1 centim in diameter. Both embryonic tissue of the allantois and adult tissue of the spleen participated in the formation of the tumor. In many cases the tumors in well advanced stages consisted of a uniform granuloblastic tissue more or less richly vascularized, in which no distinction between adult and embryonic tissues could be drawn. The study of early and intermediate stages gives, however, precise information concerning the respective rôle, assigned to each tissue in the formation of the tumor.

The lymph follicle of the adult spleen is easily recognized by its arterial vessels and by the presence of small lymphocytes, which are lacking in the allantois of the embryo. Usually follicles, which in the cultures are immediately adjacent to the allantois persist and thrive. Several types of cells constituting the follicle react in definite and differing ways. The fate of the following cells of the spleen follicle in the culture could be determined:

1. Small lymphocytes. 2. Cells forming the reticulum. 3. Stem cells of the small lymphocytes.

Numerous small lymphocytes emigrate out of a follicle into the loose tissue of the allantois. Some of them remain in the follicle. They do not show here any signs of activity but remain inert. Very rarely can a mitotic figure in a small lymphocyte be recognized. The small lymphocytes gradually die out and are ingested by adjacent cells; in their cytoplasm remnants of ingested small lymphocytes may be discovered a long time afterwards.

Phenomena of a substantially different order are observed in the two other characteristic structures of the lymph follicle, in the hemoblasts and in the cells forming the reticulum. They are of a progressive order and reveal in these cells a high power of activity. Transplanted into a loose embryonic mesenchymal tissue the lymph follicle loses gradually its dense appearance; numerous cells of the reticulum hypertrophy, become free and together with the existing stem cells of the lymph follicle form large groups of amoeboid cells. The structure of these cells correspond in all details to that of the numerous hemoblasts which simultaneously develop at the expense of the embryonic mesenchymal cells in the allantois around the culture of the spleen.

If left in undisturbed relations with its normal environment the lymphoid hemoblasts of the adult spleen would naturally develop into small lymphocytes. But now when subjected to conditions, in which an embryonic lymphoid hemoblast displays an intensive granuloblastic differentiation, the lymphoid hemoblasts of the adult spleen follow exactly the same lines of differentiation, they become granulocyto-blasts. New environmental conditions disclose in the lymphoid hemoblast of the adult spleen a potency to differentiation which was inhibited by its previous environmental conditions. The lymphoid hemoblasts of the adult spleen (and this applies as well to the mesenchymal cells of the reticulum) are morphological units, the development of which into small lymphocytes under normal conditions is but the realization of one possibility out of its larger range of potentialities.

21. *Differential factors of erythro-granulopoiesis in the embryo of mammals and sauropsids.* (With demonstration of hemotopoiesis in the yolk sac of birds.) VERA DANCHAKOFF and CLAYTON SHARP, From the Anatomical Laboratory of Columbia University.

The development of the hemotopoietic function in the mesenchyme seems to be closely connected with the development of vascular channels in more than one respect.

The first appearance of blood corpuscles is inseparably connected with the first development of vessels. Even the endothelial wall seems to become in Sauropsids, a differentiating factor in the development of the common mother cell. Again, the presence of a large sinus like venous capillary net favors highly a hematopoietic differentiation

in the mesenchyme. The effect upon the stem cells of separation by an endothelial wall, the influence exercised by the intravascular conditions within a sinus-like net are factors, which determine, together with the cell complex of the cell its further differentiation. A comparative study of the development of the first hemotopoietic organs in mammals and Sauropsids is of interest when connected with the study of its determining factors.

The first striking difference in the hemotopoiesis of these two classes of animals is the difference in localization of hemotopoiesis during a great part of their embryonic life. In mammals hemotopoiesis is localized in the liver, while in Sauropsids, it is localized in the appendages of the yolk sac. If hemotopoiesis is an activity of the mesenchyme, induced by a definite complex of environmental conditions, there must be found a striking analogy between conditions encountered in the liver of mammals and those prevailing in the appendages of the yolk sac.

It is of course easy to understand, that hemotopoiesis cannot take place in the walls of the mammalian yolk sac, for there is not space in this region for a hemotopoietic organ to develop. Why, however, is this process transferred to the liver? May the circumstance of its entodermal origin constitute a factor in the determination of this organ as a hemotopoietic center? It does not seem probable, for the definite organ of erythro-granulopoiesis associates with the bone marrow, which is not an entodermal derivative.

It has been pointed out, that the large quantity of food material, present in the yolk sac, is one of the chief factors in determining the development of erythro-granulopoiesis in this region. Is this requirement fulfilled in the mammalian liver? It is, for the umbilical veins, carrying the resorbed food from the placenta, traverses the liver. The presence of this large quantity of food can be effective, only if the blood carrying it flows slowly through large channels. Such conditions are found in the hemotopoietic organ of the birds. These conditions repeat themselves in the liver of the mammalian embryo, when the developing liver converts the lumen of the umbilical veins into sinusoids. The existence of conditions favoring the absorption of definite substances from the blood stream, seems so important, that hemotopoiesis does not occur where these conditions are absent, e.g., in the placenta whose vessels are merely thoroughfares for the blood.

If the mere opportunity for resorption, the presence of food material and favorable conditions, was sufficient for the development of hemotopoiesis, mesenchyme in the liver of Sauropsids would be induced to undergo a hemotopoietic differentiation, as it has in the liver of mammals; for the omphalo-mesenteric veins in birds carry large quantities of food material from the yolk sac and are transformed also by the growing liver into sinusoids. But, the mesenchyme of the bird liver does not develop a hemotopoietic function.

There must be an essential difference between substances carried by the blood of the umbilical veins in the mammalian embryo and those

carried by the blood coming through the omphalo-mesenteric veins of the Sauropsids. Though both are rich in food necessary for embryonic development, the blood of the omphalo-mesenteric veins of the Sauropsids has passed through a venous plexus, in the appendages of the yolk sac, which are engorged by multiplying and differentiating blood cells. These cells are the first to profit and to abstract from the resorbed food certain substances necessary for their multiplication and specific hemotopoietic differentiation.

The blood reaching the liver in the Sauropsids, therefore, has been filtered of definite substances necessary to the mesenchyme for its differentiation into erythro-leucopoietic tissue. The mesenchyme of the liver in Sauropsids, though surrounded by sinusoids, in which the resorption of food material may easily occur fails of specific development because of the lack of specific substances in this food material.

22. *Further verification of functional size changes in nerve cell bodies by the use of the polar planimeter.* (Lantern.) DAVID H. DOLLEY, Laboratory of Pathology, University of Missouri.

Kocher (Jour. Comp. Neur., vol. 26, 1916, 341) severely attacks the work of the writer on the basis of area measurements of nerve cells from undisturbed and exercised animals by means of the polar planimeter.

I have tested the planimeter method, not by averaging all cells measured from any region irrespective of their functional stage, as Kocher did, but by applying it to the same number of cells from every functional stage. Data of average diameter measurement were obtained concurrently from all series, and the two methods compared graphically. The two methods afford results absolutely identical in every detail both in size and nucleus-plasma relations, thereby verifying previous findings. The area method has no special or superior value in the case of uniformly shaped cells, though it would be well to use both methods in the case of irregular contours. Though exact in itself, the planimeter gives only two dimensions, with smaller variations than in volume calculations, and hence may be quantitatively misleading.

Kocher finds only slight variations in area size between control and exercised animals. The essential fallacy in denying any functional size differences from the results of comparative measurements when all stages are averaged together depends upon the tendency to a general uniformity of absolute size of corresponding nerve cells among animals of the same species. Further, when the third dimension is ignored, and Kocher's only reference thereto is in the heading of a table "volume expressed as square inches," it follows that there is less range of variation between functioning type sizes than if volumes were used, and the averaging tends better to equalize differences due to unequal distribution of various-sized types. Kocher's results are just what might be expected, and instead of confounding the writer in respect to functional size changes, tend only to support his induction of a uniformity of size relations as a general rule for a species.

Kocher confirms the existence of all the stages interpreted as functional by the writer. However, he admits no significance to them, because he finds them all in undisturbed as well as exercised animals. His conclusion from this of absence of qualitative differences is not, as he ignorantly thinks, destructive to me, but is the first induction I should wish to be confirmed since it throws nerve cell function solely on the fundamental quantitative principle.

He denies such quantitative differences in comparative differential counts, but since for one thing certain stages as diagnosed do not accord with his text statements and are unlikely upon the basis of relative differentiation, these counts need not be taken seriously.

Kocher's sweeping criticism falls into absurdity, being based on objective results in the above respects confirmatory of my own conclusions.

23. *'Histiocytes' and 'macrophages' and their relations to the cells of normal blood in animals stained intra vitam with acid colloidal dyes.*

HAL DOWNEY, Department of Animal Biology, University of Minnesota.

Various colloidal substances, such as aqueous suspensions of lithium carmine, of the azo dyes Pyrrolblau, Trypanblau, etc., of collargol, India ink, and of various other non-toxic colloidal substances injected into the veins of animals are taken up very rapidly and stored in the form of granules by certain cells of the hemotopoietic organs, liver, and of the loose connective tissue. Repeated injections of the same animal result in an increase in the number of 'dye granules' in the cells, and also in the number of cells which contain the dye. Cells containing the dye granules have been termed 'histiocytes' by Aschoff-Kiyono, 'pyrrol cells' by Goldman, 'macrophages' by Evans.

Schulemann ('12) believed that the dye granules represent a combination of dye with a reaction body of the cell, and also that various preformed structures, such as plasmosomes, secretory granules, etc., can be made visible by means of these dyes. V. Möllendorf, Pappenheim and Tschaschin also agree with this earlier view of Schulemann. At present Schulemann and Evans believe that the dye granules are merely concentrations of the colloidal suspensions which are located within cytoplasmic vacuoles. They are not combined with any constituents of the cell. The formation of dye granules is therefore a physical process and not a chemical union of preformed structures or receptors of the cell with the dye. In his latest paper Schulemann ('16) claims to have demonstrated the physical nature of the granules experimentally. If the results of these experiments are correct then we must agree with Evans that the fine particles of the injected suspensions are phagocytosed by the cells which are able to store the dye in the form of coarse granules. Phagocytosis, therefore, is the process which quickly cleans out the particles of the injected suspensions from the fluids of the body, and cells which phagocytose the suspended particles should obey the general laws of phagocytosis.

The cells of the circulating blood do not take up these substances, even though the latter are injected directly into the blood stream. However, histiocytes appear in the blood stream after repeated injections of the colloidal substances when the animal becomes 'overloaded,' and Aschoff-Kiyono have demonstrated them in sections of the veins of the hematopoietic organs after a single injection of lithium carmine. They do not take up the colloidal substance directly from the circulation, but become loaded with it while in the tissues, especially of the hematopoietic organs. They are never abundant in the general circulation but may be quite numerous in the veins of the liver and hematopoietic organs. The fact that Aschoff-Kiyono must resort to sections in order to demonstrate the presence of the histiocytes seems to favor the view of Mitamura and Masanori that the anesthetic, or the manipulation during the operation, or premortal agony cause the separation of endothelial histiocytes from the hematopoietic organs and their entrance into the blood stream. These authors find the histiocytes to be very rare in the veins of living animals, but quite numerous in dead animals. These facts seem to indicate that the passage of histiocytes into the blood is more or less accidental, a view which is further strengthened by the observation of Aschoff-Kiyono that they are rapidly filtered out of the circulation by the capillaries of the lung. This latter fact shows that the dye cells act as foreign bodies when they reach the blood stream, and it also accounts for the small numbers of histiocytes in the general circulation.

The few dye cells which get into the circulation in overloaded animals, and the free histiocytes of the serous cavities, *tâches laiteuses*, and many of those of the loose connective tissue are identical morphologically with the larger lymphocytes and large mononuclears of the normal blood, excepting that they contain the dye granules while the latter do not. For this reason, and because of the fact that the reticular cells, especially those lining the sinuses, and their free derivatives in the hematopoietic organs, the stellate cells of the sinusoids of the liver, and the clasmatoocytes or resting wandering cells of the loose connective tissue are the cells which show the greatest preference for the dyes, it has been claimed by Goldmann, Aschoff-Kiyono, Pappenheim, Schulemann-Evans that the dye cells are always of tissue origin. In other words, true blood cells will not take up the dyes, and we therefore have a sure method for distinguishing between blood cells and tissue cells. The free wandering cells in the loose connective tissue which take up the dyes, and those about the *tâches laiteuses* of the omentum and in the serous cavities are tissue cells although they are identical in structure with the lymphoid cells which we see in the circulating blood of normal animals.

The above authors, excepting Pappenheim, also claim that the histiocytes, both fixed and free, are closely related cells which constitute an independent cell-line which is absolutely distinct in origin and function from anything found in the circulation of normal animals.

The only worker in this field to seriously oppose the idea of the independence of the histiocytes is Tschaschin, who believes that in experimental inflammation of the loose connective tissue in vitally stained animals lymphocytes leave the vessels in great numbers and increase rapidly in size to form 'polyblasts' which take up the dye and store it in the form of the typical dye granules of the histiocytes.

The results of the following simple experiments are opposed to the theory of the independence and close relationship of all the dye cells. They also show that blood cells under proper conditions will take up the dyes as rapidly as do the so-called histiocytes.

Rabbits and albino rats were the experimental animals, and Pyrrholblau, one of the acid azo dyes, was used as the colloidal suspension.

Intramuscular injection of a 1 per cent suspension of the dye in water caused immediate migration of polymorphonuclear leucocytes to the site of injection. More polymorphonuclears were obtained if a suspension of aleuronat was injected first. When the animals were killed twenty-four hours after the injection sections of the muscle showed that the polymorphonuclears were gorged with the dye which was stored in the form of coarse granules identical with those of the histiocytes of animals stained *intra vitam* by intravenous injections of the dye. None of the leucocytes of the circulation contained the dye granules.

The most convincing results were obtained when the dye was injected directly into a segment of the femoral vein which had been isolated from the general circulation by a double ligature. The vein was tied off first and then the dye injected into the vessel between the ligatures. Twenty-four hours later the ligatured portion of the vein was removed, fixed and sectioned. It contained great numbers of polymorphonuclear leucocytes which were loaded with the typical dye granules. All of the larger lymphocytes and large mononuclears present also contained the dye granules, the latter cells being identical in structure with the free histiocytes of the peritoneal cavity of animals stained *intra vitam* by peritoneal injections of the dye. The presence of the dye in the ligatured vessel has attracted great numbers of polymorphonuclears from the vessels of the surrounding tissue or from the *vasa vasorum*, but it seems to have had no influence on lymphoid cells other than those which were already contained in the ligatured vessel. The lymphoid cells are not more numerous than would be expected in the amount of blood contained in the vessel.

The fact that polymorphonuclear leucocytes have wandered into the vessel does not destroy the value of the experiment, the most significant feature of which is the storage of dye in the form of typical dye granules by cells that are primarily blood cells, and which under ordinary circumstances do not take up the injected dyes. The lymphoid cells containing dye granules are those which were already present in the vessel, and therefore they are also blood cells.

These results seem to indicate that the reaction of cells to colloidal suspensions is not sufficiently specific to be used as an index of cell-lineage. Mechanical conditions and nature of the surrounding medium

undoubtedly play an important rôle in these reactions. In the blood stream the cells hardly get a chance to phagocytose the particles of the injected colloidal suspensions, for the latter leave the circulation almost as soon as they are injected. The presence of dye granules in the reticular cells of the lymph nodes, especially in those lining the sinus, within a few minutes after injection indicates that the suspensions pass very quickly into the lymph stream to be removed from the latter by the reticular cells.

That the reticulo-endothelial cells should show great voracity in the taking up of the dye is not at all surprising, for we know that they are among the most active phagocytes of the body. This is also true of the free cells which are cut off from the reticulum; they will phagocytose erythrocytes, bacteria or any other foreign substance about as readily as they do the injected dye.

The lymphocytes of the nodes do not take up the dye nor any other foreign substance, such as living bacteria, carmine, etc. This is because they are immature cells having relatively small amounts of cytoplasm. Tschaschin has shown that when they migrate into inflamed areas they rapidly develop into protoplasmic phagocytes which take up the dye, and the experiment with the ligatured vessel shows that the larger and more mature lymphocytes which are caught in isolated segments of the circulation, where they remain in contact with the dye for some time, will also take up the suspension. In the free circulation neither the lymphocytes nor the polymorphonuclear leucocytes take up the dye, probably because of the fact that the dye is eliminated from the circulation so quickly that the phagocytic cells are not in contact with it for sufficient length of time to be able to take it up. The mechanical agitation of the blood in the free circulation probably also has something to do with the prevention of phagocytosis.

That mechanical conditions are a factor in phagocytosis is indicated by the results of intravenous injection of ordinary carmine ground up in salt solution. The particles of this suspension are very much coarser than those of the colloids used. Hoffmann and Langerhans have shown that this substance remains in the circulation as late as 148 days after the injection, and that it does not enter the lymph nodes until 3 days after injection. Within the circulation the carmine is phagocytosed very freely by both the polymorphonuclears and the macrophages. It takes several days, however, before the last of the carmine particles are included in phagocytes (6 grams of carmine at one injection!). The entire reaction can be explained by the slow diffusibility of this substance; it is eliminated from the circulation very slowly, and the phagocytic cells of the blood have abundant opportunity to take it up.

The more diffusible colloids are also taken up by phagocytes, but isolation from the general circulation is necessary before phagocytosis can take place. A further illustration of this rule is to be found in the work of Kline and Winternitz on experimental pneumonia in animals stained *intra vitam* with Trypanblau. Polymorphonuclears contain-

ing typical dye granules were found in the alveoli, bronchioles and blood vessels of the involved lung but not in the general circulation. Injection of the blood vessels of the involved lung showed that they were cut off from the general circulation by plugs of fibrin in the capillaries. Pappenheim and Fukushi also reported dye granules in polymorphonuclears and in lymphoid cells of peritoneal exudations. Rosenthal has shown that even living organisms are rarely phagocytosed within the circulating blood. A virulent bacteria injected into the tail vein of mouse were phagocytosed by 'endothelial' cells, chiefly the stellate cells of the liver. Wandering cells of the blood became active only when the bacteria were so numerous that the 'endothelial' cells could no longer take care of them. Werigo found anthrax bacilli to disappear from the circulation within a few minutes. They were held by phagocytes in the spleen, liver and lungs.

The writer's experiments with Pyrrolblau have shown clearly that the type of phagocytic cells involved in the process of taking up the dyes depends altogether on the conditions of the experiment. According to Buxton and Torrey this is also true when living organisms are used. Typhoid bacilli and staphylococci injected into the peritoneal cavity appeared within the macrophages of the mediastinal lymph nodes within one hour. They conclude that the macrophages have seized the organisms because they were present before the arrival of the polymorphonuclears. Later the latter invaded the nodes in great numbers and seized whatever organisms were left.

The power to phagocytose may vary greatly in cells of the same type (Hektoen, Rosenow), and cells which are not ordinarily phagocytic may become so under certain conditions, as shown by Achard, Raymond and Foix, who reported a case in which the eosinophils of a pleural exudate were very active as phagocytes. Phagocytic eosinophil leucocytes have also been reported by Lattan-Larrier and Parvu, Wendenburg, and by Weinberg-Séguin.

It is evident that phagocytosis is a physiological process which is not confined to any one type of cell, and that the material to be phagocytosed which under ordinary conditions is taken up by a certain type of cell may, under slightly different conditions, be taken up by cells which genetically and structurally are quite distinct from the first type. The taking up of colloidal dyes seems to be, in many cases at least, very similar to phagocytosis as we have known it before the advent of the azo dyes. Dye granules have been reported in the following cells: reticular cells, endothelial cells, clasmatoocytes, lymphocytes, polymorphonuclear leucocytes, hepatic cells, cells of the adrenal and epithelial bodies, interstitial cells of the testis, cells of the Graafian follicles, hypophysis, plexus chorioideus, ectoderm cells of the placenta and the giant cells of this organ. The granules of some of these cells may be vitally stained preformed structures. Nevertheless, it is evident that the reaction is not specific for any one line of cells, and all attempts to classify cells according to their reactions to these dyes must be regarded as failures, especially since Kiyono, one of the most

ardent defenders of the theory of the specificity of the reaction, is forced to admit that lymphocytes of lymph nodes in the later stages of aseptic inflammation may enlarge and develop relatively more protoplasm, when it is impossible to distinguish them from the true histiocytes of the organ, and impossible to show that they do not take up the dye.

24. *A human embryo of seven to eight somites.* H. M. EVANS and G. W. BARTELMEZ, Department of Embryology, Carnegie Institution of Washington and Department of Anatomy, University of Chicago.

This embryo was obtained from an aborted ovum measuring 18.0 by 13.0 by 10.8 mm. including the villi, fixed intact in 10 per cent formalin. The age was estimated clinically as three weeks. The embryo measured 2 mm. in length in formol and belongs in the group with the Mall embryo no. 391 (described by Dandy), the seven somite Spee embryo, the 2.11 mm. embryo of Eternod and the R. Meyer embryo no. 335.

The embryo was found attached directly opposite the chorion laeve, projecting into the extraembryonic coelom at right angles to the chorionic wall, supported by a few strands of magna. The embryo lies flat upon the yolk sac, with head and tail folds rising above it. The height of the head fold may have been slightly increased by distortion in the fixative but this region could not possibly have been bent ventrally over the yolk sac as it is in the Keibel embryo of 6 somites (Normentafel no. 3), and the above mentioned Eternod specimen. The amniotic cavity is large and the amniotic duct extends along the belly stalk. In dorsal view the embryo appears somewhat like a spoon, the expanded cephalic neural folds corresponding to the bowl. The neural tube is closed from the middle of the hind brain to the level of the seventh somite. Caudally the neural folds gradually flatten out and pass over into the primitive streak at Henson's node. The neurenteric canal has already closed. It is possible to delimit forebrain, midbrain and three hind brain neuromeres since the cerebral flexure has just begun to appear, the neural crest is actively proliferating and the otic plate and ganglion are well defined. The asymmetric forebrain is bent almost at right angles to the hindbrain, the midbrain forming the knee. In the forebrain two shallow sulci can be distinguished converging rostrally: they are the earliest stage of the optic sulci yet described in man. The neural crest cells are migrating from the dorsolateral region of the folds in the midbrain and hind brain as far caudally as the VII-VIII ganglion. This is a bulbar swelling of the neural fold dorsally, lying opposite the thickened otic plate.

The pharynx is intermediate in development between that of 391 in the Mall collection and 335 of R. Meyer. Its epithelium is in contact with the ectoderm of the oral membrane, the first visceral pouch is well developed and its dorsal diverticulum touches the thickened ectoderm at one point. The second pouch is beginning to form and there is an asymmetric thyroid anlage. The hind gut extends but five sec-

tions caudal to the origin of the allantois and there is no cloacal membrane. The chorda begins near the upper end of the pharynx as a thickened ridge and is everywhere incorporated in the entoderm except in the region of Henson's node.

The heart is an almost bilaterally symmetrical tube formed by the union of the vitello-umbilical veins lying ventral to the pharynx. A pair of delicate vessels, the first aortic arches pass around in front of the first pair of visceral pouches from the bulbar end of the heart to the greatly dilated cephalic ends of the dorsal aortae. The latter have four well developed pairs of dorsal rami, the first two of which are growing in the direction of the fifth and eighth ganglia respectively. Caudally the aortae break up into a plexus on the dorsal wall of the yolk sac which plexus in turn gives rise to the umbilical arteries at the beginning of the belly stalk. More rostrally the vitelline vein is beginning to differentiate from the plexus on the yolk sac.

A comparison of the various embryos of this stage shows that the different systems of organs do not develop *pari passu* and it is impossible to arrange them correctly in a series by referring to a single character such as number of somites or greatest length. In the present case the nervous system is relatively more differentiated than any other. It is necessary to seriate a limited group of embryos separately for each organ system.

**25. Endothelium and wandering connective tissue cells as seat of origin of hemoglobin.* J. H. GLOBUS, Cornell Medical School, New York City.

In the course of an investigation of the blood elements in a large number of invertebrates, the writer selected three species of annelides as best adapted for the study of the origin of hemoglobin. The intimate relationship of this respiratory pigment with erythrocytes had lead many investigators to believe that the solution of the problem of the origin and differentiation of red blood corpuscles would also uncover the ultimate source of origin of hemoglobin. This, perhaps, holds true of vertebrate blood, but in invertebrates and, especially, in annelides it is possible to find species with hemoglobin circulating free in the blood plasma. Here the way is open to trace the respiratory pigment to the group of cells, tissue or organ responsible for its formation.

The three annelides studied show wide variations in the organization of their vascular system, while they retain great similarity in the rest of their internal structures. *Arenicula Cristata* a marine annelide, has a very highly developed vascular system, with pulsating heart, numerous large and small blood vessels, forming a net-work about the gut and sending in branches into the body wall. *Diopatra dibrah-chiata*, another marine annelide, shows a less complex vascular system, the vessels being arranged mainly about the gut and forming by their smaller branches a plexus. In *Rhyncobolus*, on the other hand, we find only a rudimentary ventral vessel, the blood fluid occilating in the hæmocoel.

In *Arenicula* one finds in the intermuscular connective tissue, a large number of giant cells loaded with brownish rounded pigment granules. These cells when fixed are found to be arrested in various irregular forms, indicating amoeboid movements. Their progress seemed to be in the direction of blood vessels. These blood vessels are surrounded by similar large pigment cells which seem to form their limiting wall, as no other tissue, fibrous or muscular is there discernible. Some of these cells are arrested by the killing fluid in the process of emptying their granular contents into the lumen of the blood vessel. Their granules when stained with iron-detecting reagents indicate a rich iron content. They also react readily to Eosin and other acid dyes in a way similar to the hemoglobin containing plasma and erythrocytes in vertebrates. Other staining reactions indicate that (in some stages) these granules are modified nuclear derivatives. In some cells there are discernible three types of granules showing a transition from a nuclear to a plasma reaction, with the indifferent stage of brownish granules intervening. Thus, it is reasonable to believe that these cells are concerned with the manufacture of the respiratory pigment.

Similar conditions exist in *Diopatra*, with the only difference that the wandering mesenchymal cells are not as numerous, and that the endothelial cells are not as large.

It is interesting to note that while in the above two species which have rather highly developed vascular channels, the hemoglobin is held in solution in the plasma, and can be traced to the cells lining the walls of the blood vessels in *Rhyncobolus* we have only a rudimentary vessel, and here the hemoglobin is incorporated in nucleated red blood corpuscles. Thus the origin of hemoglobin in this species is obscured and its development must be worked out along the lines of origin of the hematids. There is, however, sufficient ground to believe that these hematids are derived from the mesodermal layer of the wall of the gut.

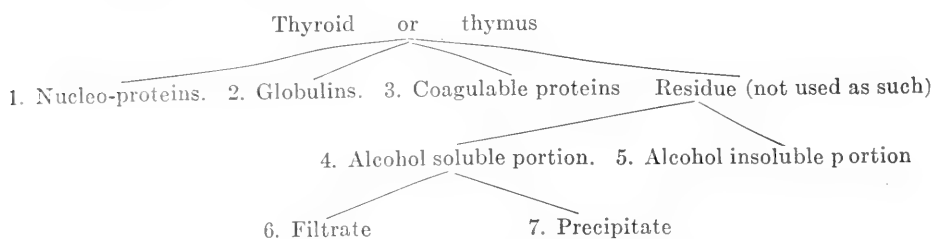
26. *Studies on internal secretion IV. Treatment of tadpoles with thyroid and thymus extracts.* J. F. GUDERNATSCH, Department of Anatomy, Cornell University Medical College, New York City.

In some previous experiments on growth and differentiation portion of fresh thyroid, thymus and other glands were given as food to developing tadpoles. At the same time the animals lived in aqueous extracts of the entire glands, some of the tissue juices, of course, going into solution. In the experiments reported here an attempt was made to study the influence upon the development of tadpoles of several distinct constituents of the thyroid and thymus. By a detailed chemical procedure each organ was split into seven products, all in aqueous solutions.¹

¹ The solutions were prepared by the Department of Experimental Medicine.

Six of these portions contained each one 'agent,' the seventh, termed the alcohol soluble portion, combined two 'agents,' precipitate and filtrate, before the process of precipitation.

The seven isolated portions were:



The nitrogen content of the solutions was determined and the same concentration used for each set of tadpoles. An ordinary meat and vegetal diet was given.

The thyroid and several of its constituents proved the most powerful stimulants for accelerated differentiation, while growth was suppressed almost entirely. When graded according to their action on differentiation the thyroid portions give the following list:

(Thyroid, entire gland, dessicated; Parke, Davis and Company)	
Thyroid nucleo-proteins.....	no growth
Thyroid globulins	
Thyroid alcohol insoluble portion	
Thyroid coagulable proteins	
Thyroid alcohol soluble portion	
Thyroid filtrate	
Thyroid precipitate.....	greatest growth
Control	

The last three products hardly showed any effect on differentiation, the tadpoles treated with the precipitate running almost parallel to the control. Tadpoles treated with the first four portions, however, metamorphosed from five to six weeks before the control, while they never reached the size of the control animals. Nucleo-proteins and globulins checked growth entirely.

Three sets of the experiment started at different ages of the tadpoles—each interval being about four weeks—showed strikingly that older animals react to the thyroid treatment faster than younger, being, of course, nearer the stage of metamorphosis. In the youngest set the nucleo-proteins required twenty days to bring about metamorphosis, in the next set six days and in the oldest four days.

It has been claimed that the amount of iodine present in the thyroid is mainly responsible for its action. It might be possible to determine this question by using the several thyroid products in the same iodine concentration. In the present experiment they were equalized as to

their nitrogen content. Graded in regard to the iodine present they range as follows:

Nucleo-proteins
Coagulable proteins and alcohol soluble portion
Globulins
Alcohol insoluble portion
Filtrate
Precipitate

This column does not run parallel with the previous one, although the nucleo-proteins and the precipitate occupy the corresponding places.

Considering the action of the thymus as a whole, viz., retardation of differentiation, these experiments furnished some bewildering results. Only two of the seven products of the thymus delayed differentiation considerably, the alcohol soluble portion and the precipitate. The rest brought about differentiation simultaneously with or ahead of the control. It is impossible at present to explain these facts. It becomes necessary to assume either that the thymus furnishes several products of different physiological properties or that five of the seven constituents of the fresh gland are inactive. The latter assumption does not seem plausible; for the mass of the nucleo-proteins alone, for instance, is far greater than the alcohol soluble portion.

Beginning with that thymus product most active in retardation a graded list of the seven constituents would read as follows:

Thymus precipitate
Thymus alcohol soluble portion.....slowest growth
(Thymus entire gland, dessicated; Parke, Davis and Company)
Thymus filtrate
Control
Thymus alcohol insoluble portion
Thymus globulins
Thymus nucleo-proteins.....fastest growth

The expectation that the corresponding constituents of the two glands would be most active in counteracting each other was not realized; for it will at once be seen that in general the serial arrangement of the thymus products is almost the opposite of that of the thyroid series.

It is impossible here to give all the growth curves. In the thyroid group the nucleo-proteins (strongest differentiator) permitted no growth, the filtrate and precipitate (weakest differentiators) allowed the tadpoles to grow to normal size in about the normal time. Thyroid filtrate and precipitate may well be considered inactive as far as differentiation is concerned. They may have other physiological properties not disclosed in these experiments. In fact, injection experiments on mammals revealed such properties, especially in the case of the filtrate. In the thymus group the nucleo-proteins gave most, the alcohol soluble portion least rapid growth. Both have their position at opposite ends of the above list showing their influence on differentiation.

*27. *The effect of feeding Sudan III to albino rats.* S. HATAI, The Wistar Institute of Anatomy and Biology.

The primary object of feeding Sudan III was to determine whether the myelin sheaths could be stained as they were formed. This attempt failed entirely but the alterations noted in the growth of the body and organs suggested the desirability of further studying these responses. The following is a brief summary of the results so far obtained by the use of 135 rats, representing 19 litters.

1. The amount of Sudan III given was 0.008 grams per rat per day. The Sudan was mixed with 1.5 cc. of olive oil and given with the other food.

2. It has been found that rats less than 30 grams in weight do not grow at all when given the Sudan III in oil, while rats more than 30 grams but less than 50 grams in weight show an increase in weight for the first few days of feeding, but if the Sudan is continued they begin to emaciate. Rats which weigh more than 50 grams appear highly resistant to this dye and it takes several weeks of feeding before they show the effects seen in the younger animals. We find no noticeable injurious influence due to the oil, when given in the quantity here used.

3. When the effect of the Sudan III is marked, the thymus and sex glands show considerable diminution, while the liver and pancreas show noticeable increase in weight. In several cases the thymus gland had disappeared completely.

4. The percentage of water in the liver and brain diminished, while that in the lungs, pancreas and blood increased. An increase in the alcohol-ether extracts was found in the liver, brain and blood.

5. As determined by a color test, a trace of Sudan was recovered from the lungs, pancreas and kidneys, while more than a trace was found in the liver. Complete absence of the dye was noted in the brain, spleen and heart.

6. All the Sudan rats were in a state of profound anemia. In one case nearly 40 per cent reduction of erythrocytes was found.

7. The present investigation shows that retardation of growth by the feeding of Sudan III can not be explained as due to a simple starvation, owing to the reduced availability of the fat after staining, as Riddle concludes, as there is clear evidence of more extensive pathological alterations such as anemia, fatty liver, nephritic kidneys, etc.

Since Sudan III fails to enter and stain the myelin, it is highly probable that this substance is not obtained directly from the ingested food, but is very probably developed in situ.

28. *The ganglionic crest and mesectoderm of the chick in relation to the closure of the neural tube.* FRANCIS W. HEAGEY, Creighton Medical College, from the Department of Anatomy, Columbia University.

The ganglionic crest of the trunk has long been known to differ from the mode of formation of that of the head. Neumayer in the Crocodilian embryo, Schulte and Tilney in cat embryos have shown that the ganglia of the cranial nerves were wholly derived from the

neuraxis without the participation of the ectoderm so that the crest was not an element intermediate between the neural tube and the somatic ectoderm. In view of this it was suggested that in more cephalic portions of the neural tube where ganglia were not formed that the ganglionic element might form a permanent constituent of the wall of the brain. In an effort to see whether there was an equivalent mode of formation in the sauropsids and what part the mesectoderm plays in the formation of the ganglionic crest I incubated and sectioned forty chick embryos, exercising great care in the determination of the age of each embryo. Weigert's hemotoxylin was used for the staining of the sections.

There were several marked differences from the mammalian type of the ganglionic crest formation. The medullary plate unlike that of the cat, showed no place of sudden transition from the neural to the somatic portions, i.e., there was no sharp neuro-somatic junction. Whereas in the mammal the cranial nerve anlage appeared as derivatives of the neuraxis in the angle between somatic ectoderm and the neural tube before the closure of the latter; in the sauropsid there was no evidence of any similar outgrowth until after the closure of the neural tube and the separation of the latter from the ectoderm. In the formation of the neural tube the amount of the medullary plate which was assimilated by the neural tube in the head region varied considerably from embryo to embryo. As a consequence the area of the surfaces of the medullary folds opposed to form the neural tube varied and the resulting stem which connected the neural tube with the ectoderm was either long or short depending upon the above conditions. Then bilateral cleavage planes appeared which separated the neural tube from the ectoderm and in so doing divided the stem into two portions, one a group of cells connected with the ventral surface of the ectoderm and the other portion was connected with the dorsal fusion point of the neural tube; from the former the mesectoderm is derived exclusively. The mesectoderm was present only from six to nine somites and in the prequintal region exclusively. It had no connection with the neural tube or relation with the ganglionic crest. Its presence depended upon the amount of the medullary plate included in the neural tube and the level of the cleavage planes through the stem of the neural tube. The portion of the stem of the neural tube remaining attached to the dorsal fusion point of the neural tube assumed different shapes depending upon the region and the relations to the cranial nerve anlagen. In the intervals between the cranial nerve anlagen it was merely a wedge of cells inserted between the dorsal extremities of the neural tube. In the region of these nerve anlagen it formed a prominent mound of cells from whose sides lateral processes were seen to project.

The cells of the dorsal fusion point of the neural tube are a part of a true ganglionic crest because of their position in the neural tube, their relation to the cranial nerve anlage and their intimate connection with

the lateral processes on the dorsum of the neural tube in the quintal and the acustico-facial regions.

Based on these facts it is reasonable to conclude that the ganglionic crest of the sauropsid like the mammalian form is inherent in the neural tube and, that the mesectoderm does not participate in the ganglionic crest or the cranial nerve anlage but contributes solely to the head mesenchyme.

29. *Maturation and initiation of development in Cumingia.* L. V. HEILBRUNN, (introduced by A. C. Eycleshymer,) Department of Anatomy, University of Illinois, College of Medicine.

The egg of the mollusc *Cumingia* is immature when shed into the sea-water, and it remains immature unless it is fertilized. Soon after sperm entrance, the first polar body is given off, and this is the first step in the developmental process. Artificially, polar body formation can be induced in any one of three ways.

In the first place, if the surface tension of the vitelline membrane is markedly lowered, it rises from the egg and maturation follows. This effect is in general produced by all substances which markedly lower surface tension, irrespective of their chemical constitution. Although many such substances were tested, only one or two failed to produce maturation, and in these cases the egg was very evidently injured by the reagent.

Secondly, if the stiff vitelline membrane of the egg is caused to absorb water and swell, then maturation also occurs. Such a swelling effect is produced by certain salts, and by dilute acids and alkalies.

Finally, if the vitelline membrane is removed by shaking, or if it is ruptured by placing the eggs in diluted sea-water, the egg can be made to undergo maturation.

All three of these treatments have one effect in common. The stiff vitelline membrane which encloses the egg is replaced by a much more plastic film. Thus in the *Cumingia* egg, as in the sea-urchin egg,¹ development can only occur if the egg is freed from the constraint of a stiff vitelline membrane. Moreover, the same types of reagents produce membrane elevation and membrane swelling in both eggs. But in *Cumingia*, unlike *Arbacia*, it is a membrane swelling rather than a membrane elevation which is the normal process.

Of the two leading theories of initiation of development, neither could be applied to the *Cumingia* egg. No evidence could be found for increase of permeability. On the other hand, the maturation process takes place normally in concentrations of potassium cyanide two hundred times as great as those supposed to check oxidations.

Although polar body formation can readily be produced by the methods just described, in the great majority of cases, segmentation does not follow. As in the sea-urchin egg, a gelatinization or coagulation can be demonstrated to precede segmentation.

¹ Heilbrunn, '15, Biol. Bull., vol. 29, p. 149; v. especially p. 183.

Thus it is apparent that the theories advanced by the writer to explain the initiation of development in the sea-urchin egg, are directly applicable to the egg of *Cumingia*. The only essential difference between the eggs lies in the fact that in the *Cumingia* egg, cortical change stimulates to maturation, whereas in the sea-urchin egg the maturation process has already been completed before the egg is fertilized.

30. *On thyroidectomy in amphibia*. E. R. HOSKINS and MARGARET MORRIS, New York and Yale Universities. Experiments performed at the Yale Zoology Department. (Presented by Dr. Hoskins.)

With due care to technique it was possible to remove successfully, the anlage of the thyroid gland, from young growing larvae of *R. sylvatica* and *Amblystoma*. The stage best suited for this experiment is that just preceding the beginning of the circulation of the blood. At this time there is no danger of hemorrhage and the chances of regeneration of the removed gland are fewer than with younger larvae. Chlorotone in salt solution was used to produce anesthesia.

The experiments are to be repeated on an extensive scale the coming season, as many more data are needed for final conclusions. The following results were obtained from thyroidectomy in 40 *R. sylvaticae* and 50 *Amblystomae* checked against an equal number of control animals.

A few of the thyroidectomized frog larvae developed abnormally shaped external gills in some of which, no circulation was to be seen. This was evidently due to injury to the vascular system. One animal developed no external gills although it lived and grew through the period during which external gills normally persist. From time to time larvae were killed and fixed. A number of them, both control and experimental died and were lost.

The operated animals grew less rapidly than the controls. Only one control and one experimental animal survived the normal period of metamorphosis. Of these the control showed hind legs two months after the operation and the other had not developed legs four months after the operation. The operated larvae showed no marked tendency toward albinism.

Serial sections were made of eight experimental larvae. The operation was seen to have prevented development of the thyroid gland in all but one case. The hypophysis as compared with that of the controls showed no changes in size or structure to be attributed to loss of the thyroid gland.

Among the *amblystomae* none developed abnormal gills. The average growth rate of the experimental larvae was less than that of the controls, but of the fourteen which were alive, after three months, the largest had had the thyroid removed. In none of the thirteen operated animals that were sectioned was there any regeneration of the thyroid. There were no changes in the hypophysis nor in the pigmentation of the skin following the thyroidectomy.

- *31. *Preliminary remarks on a collection of eleven gorilla brains recently acquired by the Division of Physical Anthropology, U. S. National Museum.* (With demonstration of casts.) A. HRDLÍČKA, Smithsonian Institution, United States National Museum.

Through fortunate circumstances the Division of Physical Anthropology, U. S. N. M., has recently acquired, from the Kamerons, a precious collection of 14 brains of anthropoid apes. Of these brains no less than 11 are those of gorillas, while 3 are chimpanzees; and of the gorilla brains, 5 are adult, the rest ranging from young to practically full grown. Of the chimpanzees, 1 is adult, 2 young.

The whole collection is in a remarkably good state of preservation, with the exception of two brains of the young, and offers unprecedented opportunities for study.

The form of the brains, the fissure pattern and the variations, are of much interest.

- *32. *The morphological basis for the dominant pulmonary asymmetry in the mammalia.* GEO. S. HUNTINGTON, Columbia University.

The development of an eparterial bronchus only on the right side, and the pulmonary asymmetry resulting therefrom, is prevalent in at least 95 per cent of the mammalian genera and species whose intrapulmonary architecture is known, and the etiological factors responsible for this condition have been much discussed.

The asymmetry has in a general sense been ascribed to the left-sided position of the heart and aorta in the mammalia and to the resulting curtailment of the left thoracic space available for pulmonary extension. The difference in the mechanics of the intrapulmonary circulation in the two lungs during the placental period, due to the retention of the dorsal terminal of the sixth left aortic arch as the Botallian duct, has also been cited as a possible cause contributory to asymmetrical pulmonary development. In the early ontogenetic stages the rotation of the stomach has also appeared as offering a bar to the caudal progress of the primitive left lung-tube.

Aeby ('80) considered the dominant asymmetrical bronchial type of the mammalia to be derived from an ancestral bilaterally symmetrical eparterial condition by the phylogenetically acquired reduction and subsequent complete elimination of the left eparterial component, the right eparterial bronchus alone persisting and producing the asymmetry. Aeby offered no morphological evidence in support of this assumption.

Such evidence was apparently supplied in 1896 by d'Hardiviller, who described in rabbit embryos of the thirteenth day a transient bronchial vesicle arising from the left stembronchus, in the same position and in the same relation to the pulmonary artery as the eparterial bronchial bud of the right side. This left eparterial bud appears at the beginning of the thirteenth day, develops into a distinct hollow epithelial vesicle, whose lumen connects by a narrow canal with that of the left stembronchus, and then retrogrades rapidly. By the end of the thir-

teenth day it is reduced to a solid epithelial button, having lost its lumen and the open communication with the stembronchus, to which it is now attached solely by a solid epithelial pedicle. By the fourteenth day it disappears entirely, leaving no trace of its ephemeral existence.

D'Hardiviller finds in this discovery the absolute ontogenetic proof establishing Aeby's hypothesis of an archeal bilateral symmetrical eparterial component of the bronchial tree, from which the dominant modern asymmetrical type, with the eparterial element confined to the right side, evolved through the phylogenetic loss of the left eparterial bronchus. The latter appears in the ontogeny for only a very short period as the temporary derivative from the left stembronchus above described.

D'Hardiviller does not mention the number of 13 day embryos in which he found this evanescent vesicle. The context of his publication implies, however, that it is of constant occurrence. No subsequent confirmation of d'Hardiviller's observation has been made.

In no embryos of the critical stages accessible to me, either of the rabbit, or of other mammalian forms (cat, rat), was there any trace of the structure in question.

On the other hand in a series of 70 adult rabbit lungs, which I examined by corrosion for the occurrence of bronchial variants, I found the cephalic pole of the left lung, normally supplied by the ascending branch of the first left ventral hyparterial bronchus, supplied in one individual by an atypical first side branch of the left stembronchus arising dorsal to the pulmonary artery and corresponding in position to the larger right eparterial bronchus.

Narath, in describing the bronchial variants in a series of 39 adult rabbits, has reported somewhat similar conditions in two individuals. The conclusion appears justified that, on the evidence at present available, d'Hardiviller's observation was made on one or more variant embryos which, if development had proceeded, would have yielded atypical adult individuals possessing the above described left bronchial variant, but that no warrant is given for the assumption that such embryonic variants have a phylogenetic significance in the interpretation of the normal intrapulmonary architectonics.

A detailed study of the topographical relations of the developing mammalian lung in the critical stages reveals clearly the reason for the prevalent right-sided eparterial development, and at the same time shows the possibility of the occasional sprouting of a homologous bud from the left stembronchus. The conditions, while strongly favoring the development of the eparterial component of the right bronchial tree, are all against a corresponding bronchial development on the left side. The possibility of such an occurrence exists, as shown by the comparatively rare variation of the left eparterial bronchus, both in the embryo and in adult individuals of mammalian forms with typically asymmetrical bronchial tree (*Echidna*, *Choloepus*, *Lepus*, *Tragus*, *Homo*), and by the normal bilateral eparterial type found in cer-

tain limited mammalian groups (some aquatic Carnivores and Rodents; some Cetaceans; the Camelidae among Ungulates, and the genus *Cebus* among Primates; *Elephas*, *Hyrax*).

Rabbit embryos of 10 mm. and 11 mm. show in transverse section the ventral wall of the œsophagus with the two vagi as the background against which the tracheal bifurcation, the stembronchi and their primary buds are placed. The pulmonary arteries accede to the ventro-lateral circumference of the trachea, along which they at first descend fairly symmetrically on each side. In approaching the tracheal bifurcation two of the extra-pulmonary structures gradually change their position relative to each other and to the pulmonary tube. The left vagus, which here is larger than the right nerve and forms a massive cord, turns ventrad. This is the expression of the rotation of the fore-gut to the right through which the left side of the gastric enlargement becomes directed ventrad. At the same time the left pulmonary artery turns caudo-dorsad in obedience to the sinistral axial twist of the heart and of its arterial pedicle.

As the result of these two rotations in opposite directions of the fore-gut and of the heart, the left vagus and left pulmonary artery approach each other and are, at the level of the tracheal bifurcation and of the origin of the stembronchi, crowded closely together. The reverse obtains on the right side. The right pulmonary artery turns more and more ventrad in descending, while the right vagus moves dorsad. An arterio-neural interval is thus opened up on the right side toward which the lateral circumference of the right stembronchus faces directly and into which it sends the bronchial bud responsible for the unfolding of the right eparterial bronchus. The latter thus comes to be placed between the right vagus and the right side of the œsophagus dorsally and the right pulmonary artery ventrally. On the left side this arterio-neural portal for eparterial development has been blocked by the approximation of left vagus and left pulmonary artery, or definitely narrowed to such an extent that it no longer affords a favorable path for extension from the left stembronchus. The first side-branch of the latter is hence forced to pass in front of the artery and thus becomes the first ventral hyarterial bronchus of the left side. This supplies by means of its large ascending branch the cephalic pole of the left lung, the same area which on the right side receives the eparterial bronchus.

The narrow interval between left vagus and left pulmonary artery might occasionally suffice for the passage of a bud from the left stembronchus contributing the cephalic portion of the left lung. In such a case, the adult variants above mentioned and the embryonic left eparterial bronchial bud described by d'Hardiviller, on which they are based, would be found. The ascending branch from the first left ventral hyarterial bronchus, usually supplying this area, would then be correspondingly reduced.

It is significant to note in this connection that in the rabbit, in which form both the adult variations above mentioned and their embryonic

anlage have been found, the cephalo-ventral pulmonary extension, forming the upper pole of the left lung, is very markedly reduced compared with the right side. The impulse to send an eparterial bud from the left stembronchus through the narrow vago-arterial interval, in spite of the small available space, would be fostered in this form by the reduced area it would be called upon to serve.

I believe that the developmental conditions just outlined furnish the adequate explanation of the prevalent asymmetry of the mammalian bronchial tree. This asymmetry is primarily founded on the different opportunity for cephalo-ventral pulmonary expansion afforded usually to the right and the left lung respectively, in consequence of the cardiac and oesophageal rotation in opposite directions. The sinistral turn of the heart and its influence on the initial direction taken by the pulmonary arteries is probably dependent in the first instance on the development of the left-sided mammalian aorta and its association with the left Botallian duct. This cardiac turn directs the left pulmonary artery dorsad and favors the caudal extension of this vessel in the left mediastinal background. By contrast, on the right side, the cardiac rotation directs the pulmonary artery ventrad, unimpeded by the retention of a communication with the systemic arterial arches, and this forward thrust carries into the caudal extension of the vessel.

The enormous mechanical force exerted by the Botallian duct on the conformation of the adult arterial pattern and heart is strikingly demonstrated in human major arterial variation by the figure-of-eight twist of a *right* thoracic aorta with the left subelavian artery as its last primary branch, arising from the descending aorta via a retained left dorsal aortic arch, with a left ductus arteriosus in place of a right, as should occur in right aortic arch development.

This cardiac rotation, which imparts the initial direction to the further growth caudad of the pulmonary arteries, is met, in its influence on pulmonary development, by the rotation of the fore-gut in the opposite direction, carrying the vagi with it. These two factors coöperate in determining the plan of pulmonary development. Whereas, on the right side the vago-arterial interval is widened as a result of these rotations and the avenue for the right eparterial expansion is opened up, the path on the left side is blocked by the approximation of vagus and left pulmonary artery. It is especially the massive trunk of the nerve which would stand in the way of a bud advancing from the left stembronchus dorsal to the artery, or would, at the most, permit its development only rarely, as a variant, supplying a limited peripheral pulmonary area.

Absolute proof of the above outlined genesis of mammalian pulmonary asymmetry could of course only be obtained by the study of the proper stages on embryos of mammalian forms possessing normally the bilateral symmetrical eparterial type of bronchial tree. This material has so far not been attainable.

I have observed, however, that in the Sirenia and in some Cetaceans and pinnipede Carnivores the heart in the adult is practically median

in position with slight, if any, axial rotation. The apex, forming the ventro-caudal point of the heart-cone (bifid in *Manatus*), is nearly in the median line, and the right and left ventricles have approximately an equal share in the ventricular area of the sterno-costal surface.

The stomach of *Phoca vitulina* is almost vertical with the omental borders approximating the median plane, in marked contrast to the transverse position of the completely rotated typical mammalian stomach, whose original left surface is directed obliquely ventro-cephalad in distension.

I am inclined to believe that the embryos of bilateral eparterial forms will show a diminished degree of both cardiac and gastric rotation, with consequent greater equality of the vago-arterial interval and a more even opportunity for the development of eparterial bronchial components on both sides.

I am deeply indebted to Prof. F. T. Lewis of Harvard University, for the opportunity of studying three rabbit embryos of the Harvard Embryological Collection (Series 155, 1327 and 1658).

These preparations, perfectly fixed, stained and sectioned, proved of the utmost value.

33. Effects of inanition and refeeding upon the growth and structure of the hypophysis in the albino rat. C. M. JACKSON, Institute of Anatomy, University of Minnesota.

Sections (usually serial) of 3μ to 5μ were made of the hypophysis from 91 rats, of both sexes. These included 44 normal (newborn to one year), 15 held at maintenance (constant body weight) for various periods by underfeeding beginning at the age of three weeks, 6 adults subjected to acute inanition and 5 to chronic inanition, and 21 young rats re-fed for various periods after being held at maintenance from the age of three to twelve weeks.

The material was fixed in Zenker's fluid, and usually stained with haematoxylin-eosin, occasionally by other methods.

Volumetric data were obtained for the parts (lobes) of the hypophysis (29 cases), and, in the pars anterior (distalis), for the vessels and stroma, and the nuclei and cytoplasm of the parenchyma (8 cases). The method used was by projection upon paper, the desired areas being cut out and weighed, and the corresponding volumes computed. Direct measurements were also made with the filar micrometer.

1. Volumes of the parts (lobes) of the hypophysis

During normal postnatal growth, there is considerable individual variation in the relative volumes of the various lobes; but on comparing the younger (newborn to three weeks) with the older (ten weeks and above) it appears that in general the pars anterior (distalis) becomes relatively larger, and the pars nervosa correspondingly smaller, the pars intermedia remaining about the same in relative (percentage) volume.

That the hypophysis is relatively heavier in the female rat is already known. The present data indicate that this is due (though perhaps not entirely) to a larger pars anterior in the female.

	PARS ANT.	PARS INT.	PARS NERV.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4 males, average.....	82.0	9.7	8.3
4 females, average.....	86.4	6.7	7.0

Thus in the female the pars anterior appears to have gained, with a relative decrease in the pars intermedia and, to a lesser extent, in the pars nervosa.

In three rats held at maintenance (nearly constant body weight) from age of three to ten or twelve weeks, the pars anterior appears slightly reduced in relative volume, the partes intermedia and nervosa correspondingly larger. In an adult rat with chronic inanition, the partes anterior and intermedia appear relatively reduced, the pars nervosa increased. In two adults with acute inanition, the pars anterior appears slightly increased, the pars intermedia correspondingly decreased, and the pars nervosa unchanged in relative size.

In several rats refed one-half week, one week, two weeks and four weeks after maintenance from three to twelve weeks of age, there is some variability, but in general a gradual return to the normal proportions in the lobes of the hypophysis, the pars anterior becoming relatively larger, the partes intermedia and nervosa smaller.

In two adult rats which were refed six or seven months after a long period of maintenance (three weeks to five months of age), although the hypophysis as a whole and the body weight are nearly normal, the relative volumes of the lobes are abnormal. The pars anterior is relatively low and the pars nervosa high. This resembles the change found after maintenance and chronic inanition, and may be a persistent effect of the prolonged inanition.

2. Relative size of the components in the pars anterior (Distalis)

In the pars anterior of the normal newborn rat, the vessels and associated stroma form 6.7 per cent by volume, increasing to 9.6 per cent at three weeks, and to 10.6 per cent at ten weeks. In young animals held at maintenance, the volume of the vascular stroma usually increases to about 13 per cent, and in adults under acute or chronic inanition to about 17 per cent. The parenchyma is of course correspondingly reduced in relative volume.

In the parenchyma of the pars anterior, the nuclei form about 34 per cent of the total cell volume in the newborn, decreasing to about 24 per cent at three weeks, and 20 per cent at ten weeks. The cytoplasm undergoes a corresponding increase in relative volume. During inanition, the loss is usually relatively greater in the cytoplasm, the

nuclei thereby increasing to 26 to 28 per cent of the cell volume in the young at maintenance, and to 23 to 26 per cent in adults with chronic or acute inanition.

From the volumetric data, the (calculated) average diameter of the parenchyma cells increases from $10.1\ \mu$ in the normal newborn to $11.9\ \mu$ at three weeks and $13.6\ \mu$ at ten weeks. In the young rats at maintenance the average cell diameter is 9.7 to $10.2\ \mu$ and in the adult starved rats 10.0 to $11.0\ \mu$. The nuclei average $5.9\ \mu$ in the normal newborn; $5.8\ \mu$ at three weeks; $6.0\ \mu$ at ten weeks. In the young rats at maintenance the average nuclear diameter is reduced, being 4.9 to $5.3\ \mu$; in starved adults, 5.3 to $5.5\ \mu$.

Direct measurements of the nuclei with filar micrometer give results in fair agreement with those above. The nuclei of eosinophiles are usually slightly below the general average. Nuclei of the pars intermedia are usually near those of the pars anterior in average diameter. For all data there is a considerable amount of variability, both in various individuals and in various parts of the gland in the same individual.

3. *Histological structure*

Mitoses. In forty cases, the number of mitoses in entire coronal sections of the hypophysis was counted. Marked individual variations occur. In no case was amitosis observed. In the newborn, mitoses are very numerous throughout, the average being 62 in each section of the pars anterior, 9 in the pars intermedia and 7 in the pars nervosa.

In the pars nervosa, mitoses soon disappear. At seven days, they are rare, and none were found later (growth thereafter consisting chiefly in the increase of intercellular substance). In the pars intermedia mitosis continues at a diminished rate, the average being 1 in each section at three weeks. At ten weeks and later, they are very scarce. In the normal pars anterior, the rate of mitosis likewise decreases, the average number being 62 at birth, 18 at one week, 7 at three weeks, 2 at ten weeks, and rare in adults.

In young rats held at maintenance from three to ten weeks of age, mitosis has nearly ceased. No mitoses were found in the partes nervosa and intermedia, although in the pars anterior they still occur occasionally, even in rats nearly dead from inanition. No mitoses were observed in the starved adults.

In the young rats refed after the maintenance period, mitoses reappear promptly in the pars anterior, the average number per section being about 2 after one-half week of refeeding, 7 after one week to two weeks, decreasing to an average of 3 after four weeks of refeeding. Mitoses were observed but rarely in the pars intermedia and never in the pars nervosa. The rate of mitosis in the refed rats therefore corresponds in general to that of younger normal rats of similar body weight.

Cell-structure. In the pars nervosa, the only change noted during inanition is a variable degree of hyperchromatism in the nuclei, which rarely may become shrunken and pyknotic.

In the *pars intermedia*, the cells usually suffer relatively little change in structure during inanition. The nuclei have a variable tendency to hyperchromatism, occasionally becoming pyknotic. The cytoplasm tends to lose its granular structure, becoming more homogeneous in appearance, often finely vacuolated. Around pyknotic nuclei, it is usually more deeply basophilic. Occasionally the cytoplasm may be greatly atrophied and reduced in volume, leaving the nuclei very closely packed.

In the *pars anterior*, the changes during inanition are variable. Some areas may remain nearly normal, while others in the same gland show extreme changes of atrophy and degeneration. The cytoplasm is greatly reduced in volume (as shown above), and is frequently vacuolated. There is a marked tendency to loss of the specific staining reactions, so that strongly chromophilic cells become weakly chromophilic or even chromophobic.

The nuclear changes in the *pars anterior* are likewise variable in extent and character. There is, however, a very general tendency to hyperchromatism, often reaching marked pyknosis. Karyorrhexis and karyolysis are rare.

Upon refeeding one-half week after the maintenance period (three to twelve weeks), the hypophysis retains the typical inanition structure, although mitosis has begun. After one week of refeeding some areas have become nearly normal, and after two weeks the normal structure preponderates. After four weeks, the hypophysis has usually become nearly normal for the most part, although more or less extensive atrophic areas may persist for indefinite periods.

34. *The later development of the lobule of the pig's liver.* (Lantern slides.)

FRANKLIN P. JOHNSON, University of Missouri.

Up until late fetal life the lobules of the pig's liver are fused together and form a continuous mass of liver cells, a condition not especially unlike that found in most adult mammals. It is in stages just before birth before any evidence of a segmentation of the liver parenchyma becomes apparent, and the completion of the formation of connective tissue septae is not fully accomplished until several months after birth.

In the separation of the liver parenchyma the liver cells themselves apparently take some part. The cells along the boundaries of the lobules become granular and stain more readily than the cells elsewhere. Soon they become arranged in parallel rows, or rather sheets, extending from one branch of a portal vein to another, and thus definitely mark out the future lobules. The rows of cells become split apart by a thickening of the reticulum between them. Collagen fibers gradually spread into this thickened reticulum from around the portal veins.

The question of growth of the lobules is of particular interest. Mall (*Am. Jour. Anat.*, vol. 5, 1906) states that "in the pig the lobule measures 0.8 mm. in embryos 4 cm. long until a number of months after birth," adding that "in the adult they are 1.4 cm. in diameter." My own observations are at variance with these. Thus I find beginning

with embryos old enough in which the lobule boundaries are easily recognized, that the lobules gradually increase in size. The following table giving the average diameter of the largest lobules demonstrates this point:

AGE	DIAMETER	AGE	DIAMETER
	<i>mm.</i>		<i>mm.</i>
229 mm.	0.35	3 weeks	0.49
254 mm.	0.43	4 weeks	0.51
1 day	0.43	2 months	0.59
3 days	0.45	Adult	1.2

That there is an actual increase in the number of lobules even after the connective tissue septae are formed appears evident from estimates of the total number of lobules based upon volumetric calculations. The additional lobules are formed by a splitting up of certain large lobules. In sections one finds evidence of this in incomplete septae, developing apparently after the manner of the earliest ones.

The conception that the lobules of the liver conform to a general shape and size can be easily disproven. If small blocks of pig liver are treated with 20 per cent nitric acid at 50°C. for several hours, the connective tissue septae are destroyed. The lobules can then be readily teased apart without injuring them. Thus seen, they present a great variety of shapes, some with rounded surfaces and borders, others with flat or concave surfaces and angular borders, some approaching prisms, pyramids and polyhedrons, but the majority are so irregular they liken none of the regular geometrical solids. In size the lobules likewise present great variations within the same liver, some being at least five or six times as large as others. In the adult pig the lobules average about 1.8 cubic mm. in volume, at two months, 0.31 cubic mm., and at four weeks, 0.098 cubic mm.

35. *Aortic cell clusters in vertebrate embryos.* H. E. JORDAN, Department of Anatomy, University of Virginia.

Aortic cell clusters were first described by Maximow in 1909 in rabbit embryos (*Arch. f. mikr. Anat.*, Bd. 73, p. 517). Minot subsequently ('12) described similar structures in human embryos of from 8 to 10 mm. length and in rabbit embryos (Keibel and Mall, *Human Embryology*, p. 523). Emmel reported ('15) aortic cell clusters in rat embryos, rabbit embryos, and in pig embryos of from 6 to 15 mm. (*Anat. Rec.*, vol. 9, p. 77). Jordan discovered these clusters in pig embryos (10 to 12 mm.) at about this same time, and reported their presence also in mongoose and turtle embryos (*Anat. Rec.*, vol. 10, p. 417). Emmel later ('16) published a detailed description of the aortic clusters of the pig embryo (*Am. Jour. Anat.*, vol. 19, p. 401). Meanwhile I had observed them also in the chick embryos of three to four days' incubation. Danchakoff had already ('07) reported similar struc-

tures in the chick (*Folia Haematologica*, vol. 4, p. 159). Aortic cell clusters would seem to be a common feature of certain early stages of vertebrate development.

All of the above-named investigators agree in interpreting the constituent cells of the clusters as hemoblasts. Minot alone (l. c.) disagrees with the otherwise unanimous conclusion that they represent endothelial differentiation products. That they are endothelial derivatives, however, rather than accretion products of hemoblasts from the circulating embryonic blood is easy of demonstration. Aortic cell clusters represent one phase of the general hemogenic capacity of embryonic endothelium.

The doctrine of the partial origin of hemoblasts from embryonic endothelium has become associated with the monophyletic hypothesis of blood cell origin (Maximow l.c.); that of the non-hemogenic capacity of endothelium with the polyphyletic and diphylectic hypotheses (Stockard, *Am. Jour. Anat.*, vol. 18, p. 227). The question of the genetic relationship between endothelium and certain cellular elements of the embryonic blood touches also the 'angioblast' theory of His. The two chief tenets of this theory are: 1) the inability of intraembryonic mesenchyme to produce blood vascular tissue, and 2) the incapacity of endothelium to differentiate normally into blood cells. Abandonment of the first tenet has been forced largely through the experimental work of Hahn (*Arch. Entwicklmech.*, Bd. 27, 1909), of Miller and McWhorter (*Anat. Rec.*, vol. 8, 1914), of Reagan (*Anat. Rec.*, vol. 9, 1915), and of Stockard (l. c.), and the morphologic studies of Schulte on the cat embryo (*Mem. Wistar Inst.*, No. 3, 1914), those of McClure on the trout embryo (*Mem. Wistar Inst.*, No. 4, 1915), and the studies of Huntington on the development of the lymphatics in amniotes (*Am. Jour. Anat.*, vol. 16, 1914). The disproof of the second tenet is the chief burden of this investigation.

The material studied includes three mongoose embryos of 5, 6 and 7 mm. respectively, a series of pig embryos of from 8 to 12 mm., chick embryos of the third and fourth day of incubation, and a series of twenty loggerhead turtle embryos ranging from the second to the thirty-second day of incubation. These embryos are variously preserved and stained, the several methods including fixation with Helly's fluid and staining with Giemsa's solution.

This description confines itself almost exclusively to the 5 mm. mongoose embryo and the twelve-day turtle embryo. This selected material is at just the proper stage of development to furnish the key for the correct interpretation of the larger aortic clusters of the 10 mm. pig embryos.

The study was approached by way of the yolk-sac of the mongoose embryo. The endothelial origin of hemoblasts can here be readily demonstrated. These observations on the mongoose yolk-sac confirm my previous findings regarding the hemogenic rôle of yolk-sac endothelium in the 10 mm. pig embryo (*Am. Jour. Anat.*, vol. 19, p. 277).

The second step involved a search for similar intraembryonic phenomena. It seemed reasonable to expect that, since the yolk-sac mesenchyme could differentiate directly into blood cells and into endothelium, and since the endothelium could subsequently transform into blood cells, then the same order of events should probably follow also in the intraembryonic mesenchyme; and further, since mesenchyme is the fundamental hemogenic tissue, and since both endothelium and mesothelium in the embryo are only slightly modified mesenchyme, then embryonic mesothelium and endothelium should both, in the only slightly differentiated condition, be capable of producing cellular blood elements.

That mesothelium can differentiate into vascular tissue has been shown by Bremer in the case of the body stalk of a 1 mm. human embryo (*Am. Jour. Anat.*, vol. 16, p. 447). Examination of the intraembryonic endothelium in the pig and mongoose revealed, in the smaller pericerebral blood channels, an occasional endothelial cell rounding up and taking on hemoblast features and finally separating from the endothelial wall; and led to the discovery and detailed study of the aortic clusters of hemoblasts, with the origin and significance of which this study is largely concerned. Moreover, investigation of the pericardial mesothelium disclosed very similar clusters, both attached to the visceral and the parietal pericardium and lying free within the pericardial cavity. Emmel has recently described comparable structures in the 12 mm. pig embryo (*Am. Jour. Anat.*, vol. 20, p. 73). Occasional individual cells can also be seen in process of separation from the visceral pericardium in the mongoose embryo.

As regards the aortic cell clusters, the 5 mm. mongoose embryo shows admirably various early stages in their origin and development, and so furnishes the key to the interpretation of the later products. And the 12 day loggerhead turtle embryo shows besides, the peculiar intravascular encapsulated cell clusters, and the endothelial strands, recently noted also for a 12 mm. pig embryo by Emmel, in a footnote to his paper (p. 407) on aortic cell clusters in mammals; and the conditions in this respect also are such as appear to solve the mystery of their genetic significance.

The aortic cell clusters in the mongoose embryo of from 5 to 7 mm. range from such as are composed of only a single cell to those composed of a score or more. Single cells or groups of two or three can be seen separating from the endothelium at any point, even along the mid-dorsal line. Larger groups are found only in the ventral and ventrolateral portions, frequently in more or less close relation to the mouths of the lateral mesonephric branches or the ventral intestinal rami. This proliferative activity of the aortic endothelium is present only in the abdominal portion of the aorta, approximately coextensive with the mesonephroi. Single endothelial cells may round up and take on hemoblast features and separate from the wall in exactly the same manner as that by which the hemoblasts are derived from the endothelium of the yolk-sac vessels and in the pericerebral vascular channels.

The process is the same in the yolk sac and the embryo, and indicates a common hemogenic capacity of embryonic endothelium.

The mongoose material shows also the initial stages in the formation of the larger cell clusters. Throughout the ventral half of the abdominal aorta, the endothelium at certain points appears to buckle into the lumen. This invaginated area may be more or less extensive, and may include a considerable portion or none of the subjacent mesenchyme. The cause of the buckling remains obscure, though the suggestion lies close to hand that it may be related to the caudal shifting of the embryonic representatives of the celiac, superior mesenteric and inferior mesenteric arteries; a process dependent in part at least upon the presence of a less rigid and less differentiated endothelium ventrally, permitting thus of an inequality of growth as between the ventral and dorsal walls or allowing for the formation of successively lower connecting vascular segments for the migrating definitive stems.

The endothelium seems to be lacking centrally underneath the cell clusters. This is explained by the fact that the larger clusters arise by an invagination of the endothelium over an area of some extent rather than by process of proliferation of one or several differentiating endothelial cells. Proximally the clusters show transition stages between endothelial cells and hemoblasts (laterally) and between mesenchymal cells and hemoblasts (centrally). Within the clusters some of the cells are in mitosis, while the nuclei of others may appear at some phase of amitotic division; and an occasional cell may show phagocytic properties. Sometimes the core of the cluster shows transition stages between endothelium or mesenchyme and hemoblasts. Many of the nuclei subjacent to the cluster appear to be at some phase of amitotic division.

The aortic cell clusters of the mongoose embryo originate from the cells of an invaginated area of endothelium; they enlarge by intrinsic growth and differentiation, not by accretions from the circulating blood. Similar clusters appear also in the superior mesenteric artery. In a 10 mm. pig embryo a large aortic cluster, 130 μ in diameter, occurs near the mouth of the superior mesenteric artery and consists of a hundred or more cells. Clusters occur also along the greater length of this definitive aortic stem.

In the twelve-day loggerhead turtle embryo, encapsulated clusters and extensive strings of hemoblasts attached to the endothelium appear in the inferior vena cava, near the point of fusion of the original paired subcardinal veins. The endothelial strands, some of the cells of which bear hemoblast features, are most probably only another aspect of the general hemogenic capacity of young endothelium. Similar strands appear also in the jugular vein. Emmel (l. c.) saw similar strands in the proximal portion of the left umbilical artery, and in the aorta of this level, in two 12 mm. pig embryos, and suggests that they may be related to the fusion of the two original dorsal aortae. In the case of the development of the inferior vena cava, the coincident fusion between the originally separate post- and sub-cardinal veins in-

volves the formation of young, less differentiated, endothelium and so offers a favorable site for hemoblast production by endothelium.

The encapsulated clusters present in this same region of the inferior vena cava may be explained as follows: Subjacent to such clusters the mesenchyme appears to be differentiating into hemoblasts; this observation may give the clue to the correct interpretation of these clusters. If the invaginating area of endothelium included a considerable portion of such differentiating (vascularising) mesenchyme, then the peripheral cells might possibly be so far outstripped in the expression of their hemogenic potentiality as to be forced, perhaps principally by reason of internal pressure from the differentiating and proliferating cells, to continue development along the line already begun, namely into definitive endothelium.

Emmel (l. c.) interprets the endothelial and mesothelial desquamation products, both cells and clusters, in terms of the stimulative effect of a pathologic factor upon the endothelium; a toxin whose source is in the degenerating cells of atrophying redundant ventral aortic rami, and in degenerating erythrocytes in the serous cavities in the case of the mesothelia.

That atrophying vascular stems are present at this stage, both in relation to the aorta, and the inferior vena cava, cannot be disputed. In the 7 mm. mongoose embryo solid regressive ventral aortic stems are especially conspicuous. At least a portion of the caudal shifting of the three large aortic rami is due to a progressive atrophy of upper portions of a connecting net of vessels. But coincident with this phase of a regressive development among the upper roots, there may possibly be a new formation of lower roots. I incline to see the cause of cluster formation in the latter possibility rather than in the former fact.

Great stress is laid by Emmel upon the structure of the atrophying rami. Some of these are occluded by intravascular collections of hemoblast-like cells, both in the 10 mm. pig embryo and in the 7 mm. mongoose embryo. With these intra-arterial cell masses some of the aortic clusters are intimately related. Emmel ascribes the presence of this intra-arterial mass to the stimulative action of a dilute toxin, presumably liberated by the regressive aortic branches. This explanation is suggested by an alleged comparable pathologic process where endothelium is believed by certain pathologists (e.g., Mallory) to be stimulated to the formation of 'endothelial leukocytes' ('large mononuclear leukocytes') by dilute toxins such as are produced by typhoid and tubercle bacilli. A more likely interpretation, it seems to me, would attribute the presence of the intra-arterial cells mass of the smaller rami to the relatively slightly differentiated character of the endothelium. The occlusion of the rami and the degeneration (karyorrhexis) of the cells would thus be a secondary effect of the constriction of the regressive atrophying vessels. In other words, the intra-arterial cell mass is not the result of the action of a toxin; but the occlusion and degeneration (and the possible formation of a 'toxic substance') are all

the related common sequelae of the shrinking of the atrophying vessel around a previously present, normally produced, mass of hemoblasts.

It may be emphasized that as regards the endothelial origin and the composition of the aortic cell clusters, and as regards the mesothelial origin of cellular elements of the serous fluids, Emmel and I are in essential agreement. But Emmel views these structures as the result of the presence of a stimulating toxin; I see in them only the expression of a normal inherent capacity of embryonic endothelium to produce blood cells. The explanation of the limited distribution of the clusters is to be found in a relationship to young or newly formed, only slightly differentiated, endothelium, rather than in a connection, with regressive blood vessels and an associated toxic substance.

All the facts seem to fit better the hypothesis that the hemogenic activity of embryonic endothelium is a normal function at a certain stage of embryonic development, than that the causative stimulus is a "toxin derived from degenerating vascular tissues."

36. *The formation of the anterior lymph hearts and neighboring lymph channels in bufo.* OTTO F. KAMPMEIER, Anatomical Laboratories, School of Medicine, University of Pittsburgh.

Because a detailed narrative of the origin and development of the lymph hearts, ducts and sinuses in anuran Amphibians will be published in a comprehensive treatise during the coming year, at this time only the main steps in the formation of the anterior lymph heart and of that part of the lymphatic channel system situated in its immediate vicinity will be presented. This can be most clearly demonstrated by a comparison of six or seven reconstructions of that region in consecutive genetic stages. For the purpose of orientation, it should be remarked that the anterior lymph heart during development occupies a position, at the level between third and fourth spinal ganglia, in the triangular territory bounded medially by myotome, dorso-laterally by epidermis, and ventrally the pronephros and its surrounding venous sinus.

Beginning in 3 mm. toad embryos (European common toad), a series of venous tributaries, following one another in metameric sequence, are formed in connection with the pronephric venous sinus and the post-cardinal vein. They are the intersegmental veins located at the intervals between consecutive muscle segments and confluent ventrally with the cardinal venous trunk, the first three joining the pronephric sinus and the remaining ones the post-cardinal. In 4 mm. embryos we have the first intimation of the future lymph heart. Associated with the third intersegmental vein near its junction with the pronephric sinus, a simple venous plexus appears, as yet crude and indefinite. In the next stage, a 5 mm. embryo, this plexus, the anlage of the lymph heart, has become better defined, and not only does it include the proximal segment of the third intersegmental vein, but it is also united by short channels with the preceding and succeeding intersegmentals, the second and the fourth. Gradually the lymph heart plexus becomes sharp-

ly circumscribed, its meshwork disappears by the expansion and fusion of its channel components, and at the time when the 6 mm. stage is reached, the lymph heart anlage is practically an uninterrupted chamber whose outlines already suggest its definite condition.

Coincident with the genetic changes of the lymph heart anlage, a more or less intricate network is established between the anterior inter-segmental veins, that is from the first to the fourth, so that the original metameric condition becomes obscured and finally lost. This plexus, which we may call a veno-lymphatic one in view of its later character, progressively (in 7 and 8 mm. embryos) loses all continuity with the pronephric venous sinus and unites anteriorly with the great cephalic lymph sinus and so becomes intercalated in the lymph system; it thus loses its venous function to assume that of a lymphatic, carrying the lymph stream from the head to the lymph heart. But the original connections of this transformed venous or lymphatic plexus with the heart do not persist to become the permanent ones; they break away from the heart at the same time when the connections with the pronephric venous sinus disappear, so that temporarily the heart is completely isolated except for its confluence on its ventral side with the pronephric sinus by means of a short venous stalk, the beginning of the anterior vertebral vein.

During subsequent stages of development, in 10 and 11 mm. toad embryos, the lymph heart and the dorsal longitudinal channel of the lymphatic plexus again are brought close together, evidently by the expansion of both structures as well as by the relative reduction in extent of the region between the dorsal surface of the tadpole and the pronephros. The approximation between heart and respective segment of the lymph duct becomes more intimate, as is indicated by the fact that the duct comes to lie in a groove-like depression in the dorsal wall of the heart. It is along this line of contact that secondarily an opening or tap is now established, simultaneously with a simple valve, between duct and heart. In the meantime a similar valve has appeared at the lymphatico-venous junction, the connection of lymph heart and anlage of the anterior vertebral vein. The presence of these valves and the pulsations of the heart, which begin approximately at this time, consequently determine the direction of the lymph flow from duct to heart and thence to vein.

Other and later developmental changes as well as a discussion of the homology between the process of lymph heart formation in Amphibia and that in other vertebrates will be considered in the larger paper.

*37. *A comparative study of the roof of the fourth ventricle.* J. J. KEEGAN, (Introduced by C. W. M. Poynter,) University of Nebraska Medical College, Omaha.

At the meeting of the association last year Weed reported a series of injections into the brain cavities of living pig embryos. Independently at that time I had made a similar series of injections into the brain cavities of living rabbit embryos. The technique employed was exposure of the embryo by a small incision through the uterine wall of the anesthetized rabbit and injection of a suspension, solution or dye into the lateral ventricles by means of the finest possible glass capillary tube and bulb pressure. The quantity injected was small and was controlled by an estimate of the rate of flow under a constant pressure. The most useful injection substances were the double solution of 1 per cent ferric ammonium citrate and potassium ferro-cyanide fixed in 10 per cent formol and 1 per cent hydrochloric acid for the precipitation of the Prussian blue, as recommended by Weed, and the 1 per cent ammonium citrate solution alone, fixed in the acid formaldehyde with the addition of potassium ferro-cyanide which caused a precipitation of the Prussian blue at the site of the citrate solution. The living condition of the embryo was observed in each case by the continuance of the heart beat at the time of immersion in the fixing fluid.

The anatomical findings from these injections, combined with a study of a large number of sectioned pig and sheep embryos and several human embryos, corroborated very closely Weed's results, showing a very early development (pig embryo 6 mm.) of a thin pavement-cell oval area in the roof of the fourth ventricle, which later becomes modified by the development of the transverse fold of the choroid plexus and extends to the area posterior to the plexus.

This area undoubtedly plays an important part in the early escape of the cerebrospinal fluid, as disclosed by the denser collection of protein coagula within the ventricle in contact with this area, its early intimate eodermal and vascular relation, its later relation to the first region of dilatation of the meningeal spaces and, in all early embryos injected with the double solution, by the collection of the precipitated Prussian blue granules as a dense mass in contact with the inner surface of the membrane.

Injection with the double solution in rabbit embryos up to the age of seventeen days showed no extraventricular spread of the fluid, which was rather surprising in view of the fact that the choroid plexus is quite well developed at this age. Even in the earlier stages before the development of the plexus there is present a rather typical cuboidal-cell layer of ependyma surrounding the membranous area, indicating a possible slight secretion at this age.

For comparison with the double solution injections, which appeared to act in the manner of suspensions or colloids, a series of injections of the ferric ammonium citrate solution alone was made with strikingly dissimilar results. In the stages before the development of the choroid plexus there was a diffusion of the solution into the loose mesenchymal

tissue about the rhombencephalon and only a faint indication of a condensation of the blue granules in contact with the inner surface of the membranous area. After the development of the choroid plexus there was a very rapid absorption into the circulation.

A similar series of injections was made into the midbrain cavity of chick embryos from the four day to the nine day stage, during which time the choroid plexus develops (sixth to seventh day). Similar to the rabbit embryos, in no case did the double solution reach the exterior of the brain cavities or enter the circulation. This was determined by removal and fixation of the entire embryo with its membranes. The same manner of condensation of blue granules in contact with a central membranous area of the roof of the fourth ventricle was noted. In the four day stage this was a rather small oval area in the center of the widely expanded tela of the fourth ventricle. In the seven day stage the tela is constricted at its center by the development of the internal ear and the collection of blue granules is most dense at a small central spot in the anterior half, with a more diffuse collection in contact with the posterior half. On section these areas appear as typical pavement-cell membranous areas similar to the mammalian embryos.

The citrate injections in the chick always diffused into the mesenchymal tissue in the region of the roof of the fourth ventricle and after the development of the choroid plexus (sixth to seventh day) very rapidly entered the circulation. At the seven day stage a considerable portion remained within the ventricle and as a very evident blue coloration in the mesenchymal tissue over the rhombencephalon and mesencephalon. In contrast with this, the double solution injection, remaining an hour in the living embryo, showed no escape from the ventricles.

In the eight and nine day chick the escape of the citrate solution was even more rapid, practically all leaving the ventricles within ten to twenty minutes. Embryos of the same age injected with the double solution and remaining alive for an hour showed no escape from the ventricle.

As this is only a preliminary report of a feature of a more extensive comparative study of the development of the tela choroidea and cranial meningeal spaces in various vertebrate types, final conclusions cannot be given, but the inferences from the material examined are that this membranous area of the roof of the fourth is non-permeable to the double solution in the early embryo stages while it is permeable to the citrate solution; that a slight escape of the cerebrospinal fluid occurs before the development of the choroid plexus; and that the collection of protein coagula and Prussian blue granules in contact with the inner surface of the membranous area represents a dialysis phenomenon of this semi-permeable membrane.

*38. *The structure of the skull of Ziphius.* JOHN D. KERNAN, JR., Anatomical Laboratory of Columbia University.

For convenience the skull of *Ziphius* is usually described as consisting of the rostrum and the cranium proper, the line of division being drawn tangent to the rostral borders of the maxillary tuberosities. It is well to note, however, that structurally any such division is arbitrary. The rostrum expands at its base both in the vertical and transverse diameters. The transverse expansion, effected by a broadening of the maxillae, reaches its extreme in the region of the maxillary tuberosities. So their rostral borders indeed furnish a valid line of demarcation between rostrum and cranium in the transverse plane. On the other hand, the vertical expansion, formed in the dorsal aspect of the skull by the premaxillae, on the ventral aspect by the broad pterygoids, extends much further caudad and reaches the occipital. Thus it is difficult to settle on a point where rostrum ends and cranium begins.

The rostrum is long and exceedingly massive in its structure. It is of interest to consider the mutual relations of the rostrum and cranium. The former is in reality merely the apical portion of a pyramidal mass which rests its base against a ring formed by the supraoccipitals, parietals and the basisphenoid. The junction of the occipital ring and rostral base results in the formation of the great transverse crest of the cranium. From the crest, both caudad and rostrad, the bone falls away toward the foramen magnum on the one hand and rostrum on the other, so establishing the caudal and ventral walls of the cranium. It is the wide expansion of the rostrum at its base which results, in spite of the great length of the structure, in the firmest security against all manner of strains.

In the vertical plane forces are transmitted from rostrum to the occipital ring chiefly through the massive frontal portions of the premaxillae. It must be noted that the chief strength is not gained through direct contact, for only the right premaxilla reaches the crest of the frontal, and that by a narrow process. Between premaxillae and the frontals are interposed the massive nasal bones, and it is through them that thrusts must be in the main transmitted. The surfaces of contact between nasals and premaxillae are broad and the articulations are of great strength.

In union with the premaxillae, the maxillae turn dorsad and reach the transverse crest. This portion of the maxilla, however, is merely a thin sheet of bone and can contribute no great strength in this direction.

On the ventral aspect of the skull the lines of the transmission of force pass through the pterygoids. These bones have a broad articulation with the base of the rostrum, chiefly with the palates which are interposed between them and the maxillae. There is also a small direct articulation with the maxilla and a very slight contact with the vomer. The union then is one of great strength. Caudally the pterygoids reach the bases of the basioccipital processes of the occipital region.

The ventral brace thus formed is much less firm than the dorsal, since the pterygoids are less massive than the premaxillae, and the structure of the bone is less dense. Moreover, dorsal strains are transmitted through a continuous line of bone, whereas the ventral strains must pass through three articulations. We may conclude then that strains in this direction are not so severe as those in the opposite. However, in the adult skull provision for greater firmness is made by the expansion laterad of the borders of the pterygoids which thus secure a very firm hold on the base of the skull.

Lateral strains are provided against by the expansion of the base of the rostrum in the transverse plane. This is effected, as already stated, by the spreading out of the maxillae. Through them forces reach the occipital ring along two lines. One is an inner, along the lateral cranial wall, formed chiefly by the parietals, which articulate directly with the exoccipitals. There is in addition an outer line of transmission through the postorbital processes of the frontals to the jugal processes of the squamosals, and hence to the exoccipitals. That the lines of greatest strain lie through the lateral margins of the bones is at once evident from their formation. Mesally both the maxillae and the frontals are thin sheets which lie in contact and form an articulation of great strength preventing displacements indeed, but offering no great resistance to compression. Their lateral margins on the other hand are thicker. The frontals form the massive orbital processes. These thrust forward strong preorbital processes which are locked to the maxillae by the lacrymals. Lighter postorbital processes reach the jugal processes of the squamosals which interlock with the exoccipitals.

Additional security is given to the rostrum by the character of the articulations of the bones forming in with those of the cranium. On the dorsal aspect the chief of these is that between maxilla and frontal. Both of these bones expand into broad sheets of bone, deeply concave rostrally, the maxillae fitting into the concavity of the frontal. The premaxillae embrace over the mesal margins of the maxillae and secure this border to the nasal and frontal. On the ventral aspect the pterygoid has a broad contact with the base of the rostrum and spreads over the base of the cranium to the basioccipital. Two characteristics then distinguish these articulations, their breadth and a certain amount of interlocking.

To sum up, the relation of the rostrum to the cranium proper, depends for its security on the expansion of its base, and the broad interlocking character of its articulations with the cranium. The central feature of the cranium proper is a great transverse crest, the core of which is formed by the frontals; against it in front rests the base of the rostrum. Its caudal face supports thrusts from the condyles through the supra- and exoccipitals. These bones are reinforced by certain thicknesses which may here be noted as follows: A dense ring of bone above the foramen magnum; a thick crest seen on the inner surface of the skull which passes in the midline from the foramen magnum to the

transverse crest of the skull; two heavy ridges from the caudal ends of this crest running in a latero-rostral direction to the lateral terminations of the transverse crest. Thus the central ridge and the two lateral ridges make together three re-inforcing lines of bone, which transmit the thrusts of the vertebral column to the great transverse crest of the cranium.

39. *The laws of bone architecture.* JOHN C. KOCH, (introduced by Dr. Warren H. Lewis,) Department of Anatomy, Johns Hopkins Medical School.

Wolff's law of the functional form of bone and the functional pathogenesis of deformity has had as its sole mathematical basis, the analogy first noted by the mathematician, Culmann, between the position of the trabeculae seen in frontal, longitudinal sections through the head and neck of the human femur, and the paths of the maximum tensile and compressive stresses computed for the Fairbairn crane carrying an over-hanging load somewhat as does the upper femur. The Fairbairn crane analyzed by Culmann was assumed to be solid, with a circular cross section and an outline roughly corresponding to that of the human femur with both trochanters removed so as to present a smooth, curved form. The paths of the maximum internal stresses computed in this solid crane (a body entirely different from the femur in shape and in the disposition of the material) were assumed to explain the position of the trabeculae in the upper femur, and to be conclusive proof that the trabeculae in bone are laid down in exact accordance with mathematical laws. Although suggestive, such analogy has never been accepted as conclusive mathematical proof of the law formulated by Wolff.

Wolff's law, though originally based upon faulty evidence from a mathematical point of view, is proved for the normal, human femur by the careful mechanical analysis of the inner structure of the femur; and other important relations between the structure and the stresses due to the preponderant load on the femur-head, are shown by the study presented in this paper.

That human bone obeys the laws governing elastic bodies when carrying a load has been demonstrated by numerous investigators. Therefore the laws of mechanics applicable to elastic materials also hold for bone. After a preliminary study of some thirty femurs, the writer undertook the analysis of the mechanics of the femurs from a 35-year-old subject, who weighed 200 pounds and was in good health at the time of his death by accident.

The object of this work was the exact mechanical analysis of the structure of the normal, whole femur and the determination of the relations between structure and function at every point. The femur was studied in detail in much the same manner as a structural element of a machine or a member of a truss, where the cross section varies greatly from point to point.

The left femur was used for the purpose of studying the bone in longitudinal section, the right femur was used for the analysis of the transverse sections which were made at intervals of one-fourth inch measured along the axis of the bone. The shape of these transverse sections was extremely variable and conformed to no geometrical forms, so that the formulas of calculus could not be applied directly to the mechanical analysis of these sections. Accordingly each transverse section was covered by a series of squares formed by two series of parallel lines at right angles to each other, drawn at intervals of one-twentieth of an inch from the center of gravity of the section. In this manner the area of the compact and the spongy bone was accurately found by the summation of the squares covering these areas: the integration of the product of the area of each strip one-twentieth of an inch wide by its distance from the neutral plane gave an accurate value of the statical moment of the section: the integration of the product of each strip one-twentieth of an inch wide by the square of its distance from the neutral plane yielded an accurate value of the moment of inertia of the section analyzed. These integrations are practical applications of calculus and are absolutely necessary for a mathematical analysis of any structure subjected to bending stresses similar to those in the femur. These calculations were made in detail for the femur at intervals of $\frac{1}{2}$ inch in the upper and lower extremities and at intervals of one inch in the shaft. The total number of calculations required in the preliminary studies was about 50,000, and, in the final analysis of the normal femurs studied in this paper, an additional 70,000 calculations were necessary for the analysis of the inner architecture and the computation of the maximum stresses produced in the femur by the load on the femur-head.

An assumed load of 100 pounds was considered as acting on the femur-head in the same direction as the weight of the body, under normal conditions, and the axial load, vertical shearing force and the bending moments were computed for each section in accordance with the principles of mechanics. The amounts of the stresses were then computed at the sections of the femur which had been previously analyzed. As the intensity of the stresses varies directly with the load assumed, the stresses due to any load can be determined by simple proportion once the stresses have been found for a given load.

The load on the femur-head in the normal standing position is 0.3 of the body weight; in walking, the weight carried by the loaded femur-head is approximately 0.8 of the body weight: in running, the dynamic effect of the sudden application of the body weight produces stresses twice as great as in walking, or the effect is the same as that produced by a load double the static load of walking, or 1.6 times the body weight. For the specific case analyzed, the body weight being 200 pounds, the stresses in the loaded femur due to standing, walking and running are those due to a load on the femur-head of 60, 160 and 320 pounds, respectively. Having determined the stresses in the femur for a load of 100 pounds on the femur-head the stresses for standing, walking and running are found by simple proportion.

The inner architecture of the femur is shown by this analysis to be so arranged as to resist economically the stresses produced by the preponderant load, which is that of the body weight on the femur-head in running. The spongy bone in the head and neck of the femur is shown to be arranged in the paths of the maximum tensile and compressive stresses in this region and thus resists most economically these stresses. The spongy bone in the upper femur is well adapted for resisting the shearing stresses which are greatest in the head and neck of the femur. In the shaft the greatest stresses are due to the bending action of the load on the femur-head, which are most effectively resisted by placing the material at a distance from the axis of the bone: in this region the femur is hollow, thus securing efficient resistance to the bending action. At the lower end of the femur the large expansion of the femur by the gradual transition from the compact bone of the shaft to spongy bone in the lower end is made with but a slight increase in the actual amount of bony material used, although the stiffness is more than doubled and the hinge-action of the knee-joint is made very strong against lateral bending. Thus the compact and spongy bone everywhere act in unison to produce an internal structure adapted to the character of the predominant stress at any given point.

The analysis of strength as outlined agrees with the actual breaking-strengths of femurs made by Messerer for loads on the femur-head and also for cross-breaking loads. Statistics of the location of fractures in several independent series of fractures of the femur agree closely with the proportionate distribution of fractures according to the laws of probability, as applied by the writer to the femur.

The writer believes that the evidence presented warrants the following conclusions:

1. The normal external form and internal architecture of the human femur results from an adaptation of form to function.
2. The proportions of the femur are everywhere such as to show a definite mathematical relationship between the body weight and the internal structure of the bone: there is a definite relation between the structure and the stresses at every section.
3. Spongy bone is homogeneous with compact bone as a structural material and differs from it mechanically only in possessing smaller strength approximately in proportion to its relative density as compared with compact bone.
4. The femur has a factor of safety of 5.68 for the stresses due to running, 11.36 for walking and 30.30 for standing.
5. The structure of the femur is based upon the mathematical requirements of mechanics and the inner architecture is such as to produce great strength with a relatively small amount of material; the material is arranged to correspond with the stress requirements existing at every section.
6. The adaptation of form to function, proved mathematically for the normal, human femur by exact mechanical analysis, is the general law of normal bone.

7. The thickness and closeness of spacing of the trabeculae in bone varies directly with the intensity of the stresses transmitted by them.

40. *A comparison of the Herzog and Strahl-Beneke embryos.* (Lantern.)

FRERERIC T. LEWIS, Harvard Medical School.

The Herzog and Strahl-Beneke embryos are of particular value to embryologists since they are the youngest human embryos of which satisfactory drawings of serial sections have heretofore been published (22 sections of the Herzog embryo and 60 of the Strahl-Beneke specimen). Of the Peters embryo, apparently only a single section is thus available, which has been reproduced everywhere; and although 30 sections of the Bryce-Teacher specimen have been published, they are fragments defying interpretation, at least apart from the specimens themselves. Bryce and Teacher estimate that their embryo is three days younger, and Peters two days younger, than the Beneke specimen; but that these figures are merely approximations is indicated by the fact that Bryce and Teacher consider Peters embryo to be thirteen and one-half to fourteen and one-half days old on p. 53 of their work, but fourteen to fifteen days on p. 59. Although the difference in age of the four specimens under discussion is perhaps not greater than three days, there is the widest difference in the amount of information available about their structure. The Strahl-Beneke and Herzog embryos are the ones to which at present we must resort for an approximately adequate description.

Herzog's embryo is not now in a good state of preservation and its reconstruction is in part a matter of restoration. Thus the detached tube in the body-stalk, which was originally interpreted as an allantois, is clearly an amniotic duct. The place where it was torn off from the amnion can be definitely located. (This fundamental correction, reported at the 1913 meeting of this association, involves a change in labelling rather than any other modification of the model figured by the writer in Keibel and Mall's *Embryology*, vol. 2.) The Strahl-Beneke embryo is in far better condition, and helps to explain the Herzog specimen, whereas the latter is the most interesting commentary on Strahl and Beneke's important publication. This was appreciated by Strahl and Beneke who expressed regret that Herzog's paper was received too late for them to make a detailed comparison between his sections and their own, and they merely note that his sections differ in many respects very strikingly from theirs. We can now supply such a comparison and find that the embryos are so nearly alike that they may be said to establish the essential features of a certain early stage in human development. The conditions in younger human embryos are still subjects for diagram and conjecture.

41. *Further observations on the longitudinal muscle of the pig's colon.* P.

E. LINEBACK, Atlanta Medical College, Emory University.

The longitudinal muscle of the pig's colon develops from an accumulation of myoblasts, which are earliest seen in a 39 mm. embryo, at the

mesenteric are, and rapidly spread around the circumference of the bowel. The mesenteric are also first shows fully formed muscle fibers, which are grouped into a crescentic form, and from the tips of the crescent growth extends laterally till the whole circle becomes changed into a layer of muscle fibers; completed at 140 mm. All the while the crescentic thickening is the most conspicuous part of the muscle, a condition in harmony with what F. T. Lewis finds in the human embryonic colon.

Up to the 140 mm. stage no accumulations of fibers appear which can be interpreted as taeniae. After this time two thickenings develop in the muscle, one at either side of the intestine, equidistant from the mesentery, which receive the greater number of mesenteric vessels, and become the permanent taeniae; the only bands which the pig's colon possesses. The mesenteric are never becomes a taenia, except in the caecum where it extends from the tip of this pouch to the ileum, at the line of attachment of the ileocaecal membrane.

42. *Observations on the effect of Madder-feeding, especially with regard to deposits of calcium-salts.* C. C. MACKLIN, Johns Hopkins Medical School, Baltimore, and Wistar Institute of Anatomy and Biology, Philadelphia.

It has recently been shown that the physical and chemical processes underlying the formation of normal bone and of pathological calcific deposits are similar, if not identical. Furthermore, it has been known for many years that the dyestuff, madder, when fed to animals, will specifically stain the calcium-salts of bone which are being laid down during the time that the coloring matter is present in the circulating blood, and that this is true for the callus formed in the repair of injuries, as well as for the bone of normal development. It seemed reasonable, therefore, to suppose that the same staining reaction toward the dyes of madder would be evidenced by calcaerous tissue of a pathological character as is shown by developing bone. Accordingly, madder was fed to animals which possessed calcium-salt concretions of different kinds, and the results were studied. In general it may be said that the behavior of pathological calcium-salt deposits toward the dyestuffs of madder was identical with that of bone.

The work was carried on principally with the white rat. Experimental calcification of the kidney was initiated in the well known way by unilateral ligation of the renal vessels. After a short time the kidney, which is revascularized through the adhesions of the surrounding peritoneum, becomes the seat of a process of calcification, so that upon cutting through the organ numerous hard nodules of calcium salts are found in its interior. During a part of the time when this deposit of calcium-salts was forming madder was fed, with the result that the deposit was stained. Not all of the calcium-salt aggregation was colored, however, the nodules being composed of both white and red granular masses. These latter were probably laid down during the time that madder was being fed to the animal. Thus the picture

presented by such a stained concretion recalls that of madder-stained growing bone.

Madder was fed to a young rat which presented a double cataract, with the result that a distinct pinkness of the eyes was noticed in three days. The animal was killed after six days' feeding, and the lenses cleared in oil of wintergreen. It was then seen that the red staining was at the periphery. This was also evident in the sections. Most of the calcific deposit was unstained, the red areas representing the part of the cataract formed in the six days during which madder was being fed. Normal lenses do not stain with madder, even after five months' continuous feeding.

From these results it is believed that all pathological calcific deposits in process of formation will stain with madder.

Since calcium-alizarate, an insoluble precipitate of alizarin, has been shown to be responsible for most of the staining of bone in the madder-fed animal, it may also be regarded as the principal staining agent in the case of the pathological concretions.

This identical staining reaction towards madder-dyes on the part of both developing bone and developing pathological deposits of calcium would seem to strengthen the conception that the processes involved in their formation are largely identical.

It was noted that the dyes of madder pass easily through epithelia of different characters. The observation that the bones of the fetus are stained *in utero* by feeding madder to the mother was confirmed, and it was also noted that the milk of the madder-fed mother was colored pink, and readily stained the bones of the young suckling animal. Furthermore, madder is freely excreted by the kidney. The urine is yellow or orange when passed, but this color is turned to a red upon the addition of an alkali, thus indicating the presence of alizarin. Madder-dyes may be absorbed by serous membranes and excreted by them. Proof of the former statement is afforded by the staining of the bones in the usual way following the introduction of a sterile madder-decoction into the peritoneal cavity. The latter assertion is illustrated by the pink color shown by the hydrocele fluid of an animal fed with madder.

In animals fed for three months continuously with madder the cartilage was permanently stained red. Transient staining of the lining of the cardiac end of the stomach was noted even after only a few days' feeding. This coloration was found in the superficial layers of the keratin-like material which covers the squamous epithelium in this region. It is due to direct contact with the dyestuff.

Madder-feeding was without apparent effect on growth and reproduction.

43. *Influence of heat on the eggs of Cumingia.* MARGARET MORRIS, Osborn Zoological Laboratory, Yale University. (By invitation.)

The following report is a continuation of the cytological study of the influence of heat on the eggs of *Cumingia*. In the report made last

year, it was shown that if the eggs of this mollusc are subjected to certain temperatures they undergo a sort of self-fertilization. The first polar nucleus is separated from the egg nucleus, but is retained in the cytoplasm of the egg, and the two nuclei fuse to form a cleavage nucleus. In the ensuing cleavage the form and number of the chromosomes is abnormal. Instead of 36 long threads such as are present in the normal egg, there are 50 or 60 short rods. It was supposed when the retention of the polar body was first observed that this process would result in the restitution of the normal chromosome number, but though the normal amount of chromatin is undoubtedly present, the number of chromosomes is much larger than the diploid number.

It has been found in more recent experiments, that if the eggs are fertilized and subjected to heat immediately after fertilization, the chromosomes of the first polar spindle behave as they do when the unfertilized egg is heated. They divide, as in the normal egg, and go to the poles of the spindle, where they form a number of small vesicles. These fuse gradually and two large nuclei are formed. This takes place without any cytoplasmic cleavage, so that here, as in the parthenogenetic egg, the egg nucleus and the polar nucleus are both present. In the meantime, however, the male pronucleus has undergone perfectly normal development, so that the egg contains three nuclei instead of two. Each of these nuclei contains the haploid number of chromosomes: they come to lie close together and fuse in most cases to form a single cleavage nucleus. In some instances the male and female pronuclei do not fuse before the cleavage spindle begins to form, but there is never any discarding of nuclear material. The first cleavage spindle, then, should have a plate of 54 chromosomes or three times the haploid number. Here, as in the case of the parthenogenetic eggs, the theoretical expectation is disappointed. The first cleavage spindles have about 60 chromosomes—one plate that has been counted shows 62, another 58. Although the number is about the same as that found in the parthenogenetic eggs and the outlines of the chromosomes are similar, a great difference in size is noticeable, for the chromosomes of the fertilized egg are much larger than those of the parthenogenetic one. Evidently a larger amount of chromatin is present in the fertilized egg divided into about the same number of bodies.

It was thought that perhaps the abnormal shape and number of the chromosomes in these eggs was an effect of heat which would be reproduced in normally fertilized eggs which had formed polar bodies. If this effect is one of heat only, such eggs should show equatorial plates like those of the parthenogenetic (self-fertilized) eggs. Experiments were made in which the eggs were fertilized and allowed to form polar bodies, and heated before the first cleavage had taken place. The cleavage spindles in the eggs treated in this way show a condition entirely different from that of the parthenogenetic egg. The chromatin is present in the form of large irregular masses which give the spindle an appearance similar to that of the first maturation spindle of the normal egg. The presence of the two polar bodies shows, however, that we have here an abnormal cleavage spindle.

The amount of chromatin present in the egg is an important factor, for it has been shown that eggs having less than the diploid number of chromosomes are incapable of development. The addition of extra chromatin, on the other hand, is not injurious to the egg, for those that have three times the haploid number develop fairly regularly. The number of chromosomes present in cleavage varies under experimental conditions. It is interesting to note that the mean about which this number varies is not the diploid number, but is nevertheless the same in eggs which have the diploid and in those which have a triploid amount of chromatin.

*44. *Studies on the mammary gland.* J. A. MYERS, Institute of Anatomy, University of Minnesota.

1. *The fetal development of the mammary gland in the female albino rat*

Henneberg ('00) made a careful study of the development of the mammary glands in the albino rat from the earliest appearance of the glands through the conditions found in 16 day fetuses. Also the postnatal (birth to 10 weeks) development of these glands has been investigated (Myers '16). Heretofore the developmental conditions between 16 day fetuses and newborn rats have presented a gap in our knowledge of the mammary gland. The object of the present investigation is to fill up this gap, thus completing the history of the mammary glands in the albino rat to ten weeks of age.

In 17 day and 2 hour fetuses slight mammary pits appear on the surface of the skin over the mammary gland areas forming the typical 6 pairs. The primary ducts are solid epithelial invaginations measuring only about 0.05 mm. in length. Secondary ducts have not yet appeared.

Wax reconstructions and serial sections show very definite mammary pits in 18 day 9 hour fetuses. The primary ducts remain unbranched except in the abdominal glands where short secondary ducts appear. A small end-bud is present at the terminal end of each duct.

At 20 days, 6 hours the mammary pits are still present but in the deepest part of each pit is a small eminence surrounded by a shallow furrow. The eminence corresponds to the future nipple. Deep to the shallow furrow there is a short ingrowth of epithelium which forms the epithelial hood described in postnatal stages. Secondary ducts are present in all of the glands. In the abdominal and first inguinal glands tertiary and terminal ducts were observed. The terminal ducts present well developed end buds. In many of the terminal ducts lumina are quite well formed. The lumina begin at the distal end of the tertiary ducts and terminate about 30-40 μ from the distal end of the terminal ducts.

2. *A comparison of the mammary glands in male and female albino rats*

In the postnatal stages observed mammary gland nipples have not appeared in the male rat, although as previously shown they are very conspicuous in the female.

Male fetuses of 18 days 9 hours present no mammary pits. Instead there is corresponding to each gland a slight eminence covered with thickened epithelium. The ducts are similar in size and form to those of the female fetuses of the same age.

At 20 days and 6 hours there is neither pit nor eminence over the mammary gland areas but the ducts come directly to the surface, the epithelium of which is slightly thickened. The epithelial hood found in female fetuses of this stage is absent. The ducts of the male have branches and lumina corresponding with those of the female.

45. *The history of the eye muscles.* (Lantern.) H. V. NEAL, Tufts College.

The attempt is made in this paper to demonstrate on the basis of embryological evidence the exact homology of the first three permanent myotomes of *Amphioxus*, *Petromyzon*, and *Squalus* and to describe the more important stages in the phylogenesis of the eye muscles.

The evidence is presented for the first time to support the assertion of Dohrn ('04) and the writer ('07) that the second as well as the third myotome participates in the formation of the external rectus muscle. In the light of the evidence given the familiar text-book formula for the ontogenesis of the eye muscles should be amended as follows:

From the first myotome (pre-mandibular head-cavity) arise the muscles innervated by the oculomotor, viz., the *Mm. recti superior, internus, and inferior*, and the *M. obliquus inferior*;

From the second myotome (mandibular head-cavity) develop the *M. obliquus superior* and the ventro-lateral portion of the *M. rectus externus*;

From the third myotome (hyoid head-cavity) arises the dorso-medial portion of the *M. rectus externus*.

46. *The early morphogenesis of the thyroid gland in *Squalus acanthias*.*

E. H. NORRIS, (Introduced by C. M. Jackson) Institute of Anatomy, University of Minnesota.

The anlage of the thyroid gland in *Squalus acanthias* makes its appearance in embryos of approximately 4.0 mm. in length, as a solid epithelial bud from the floor of the posterior pharynx. This bud, which is at first little more than a thickening of the entodermal lining of the pharynx, is placed just ventral to the point at which the oesophagus leads from the pharynx, and in the region inferior and caudal to the ventral extremities of the first two gill pouches. The bud increases in size and extends caudally. It assumes a pedunculated form, being suspended from the floor of the pharynx by a rather extensive but very narrow neck. By the time the embryo has attained a length of 19 mm.

the gland has severed its connection with the pharynx, and has the form of a column with rounded ends and whose cross-section is ovoidal. At 28 mm. the gland has once more changed its form, appearing at this time as a relatively thin diamond shaped plate. At 36 mm. the gland has increased remarkably in length and is divisible into an anterior, diamond shaped corpus and a posterior, elongated, narrow cauda. During the previous stages the gland has presented surfaces which have been quite smooth, but at 48 mm. the surfaces begin to lose their smoothness and by 100 mm., which approximately marks the end of the prefollicular period, the surfaces have been greatly altered by the development of great numbers of small ridges and furrows.

While these changes have been altering the external gross form of the gland, various other changes have been in process within the gland mass. Two of these deserve particular note. During the period immediately preceding the separation of the gland from the pharynx a large amount of pigment has been deposited in the gland. Although this pigment is not confined to any particular part of the gland, by far the largest part of it is found in the narrow and constricted neck which suspends the gland from the pharyngeal floor. The pigment disappears after the gland has gained its independence from the pharynx. The second process is that which has to do with the development of completely closed cavities within the gland mass. It is important to note that these cavities are quite distinct from the follicular cavities which develop later. They finally open to the outside and are invaded by the vascular mesenchyme. These cavities are produced by the vital activity of the cells which rearrange and redistribute themselves about the forming spaces. By the appearance of these intraglandular spaces the gland is transformed into a number of irregular epithelial plates, two cells in thickness, in which the primary follicles develop.

These findings, as regards the development of the primary follicles from the two-celled epithelial plates, are of particular interest in the light of recent work upon the early development of the human thyroid.

47. *On the position of the vitelline arteries in human embryos.* (Lantern.)

JAMES W. PAPEZ, Atlanta Medical College, and FREDERIC T. LEWIS, Harvard Medical School. (Presented by Professor Papez.)

This paper is a continuation of the study of the mesenterium commune reported at the last meeting of the association. The relation of the mesenteric artery to the rotation of the intestine can now be more fully considered. Broman, in 1914, declared that the proximal part of the definitive vitelline artery arises, as is well known, by the fusion of a pair of ventral aortic branches. Evans ('12) reviewed the evidence that instead of fusing, either the right or left members of an original pair may shift to the median line and become the persistent vessel, and he concluded that "the question is an open one." The formation of the very short median stem which by great elongation becomes the superior mesenteric artery, requires an anastomosis or par-

tial fusion between the right and left vessels, but not necessarily a complete fusion. Sections of the successive median roots of the mesenteric artery in a single embryo seem to show that sometimes the vessels fuse from the aorta outward, and sometimes one member of a pair enlarges as its mate becomes obliterated. After the short median vitelline trunks have been formed and have anastomosed antero-posteriorly to make the final superior mesenteric artery, that vessel increases rapidly in length and caliber. In an embryo of 4.6 mm. its trunk divides into several branches which encircle the intestine and re-unite as they proceed to the yolk-sac. The vessels crossing the intestine are remnants of the considerable row of vitelline arteries of earlier stages, and by persistence on one or the other side, they would cause the mesenteric artery to cross on the left or right of the ileum respectively. Usually the persistent vessel crosses the intestinal tube on the right side as seen in a 7.5 mm. embryo.

At 10 mm. the intestinal loop has dropped downward over the artery, which then appears below the ileum; and at 14.5 and 22.8 mm. the artery is seen on the left side of the small intestine. Nevertheless it is morphologically on the right side, as is made apparent by undoing the effects of intestinal rotation. In all these stages it does not become free from the mesentery until it is about to cross the intestinal tube. (The same is true of the vitelline vein, but since the latter crosses the duodenum, it has a long course as a free vessel within the abdominal cavity).

In an embryo of 44.3 mm. the intestines are entirely within the abdomen. The vitelline artery has now become free from the mesentery for a considerable distance before reaching the ileum. Thus it comes out from the left side of the mesentery as a strand which extends to the umbilicus. Owing to further rotation, the intestinal tube seems to encircle it completely; but again by undoing the intestinal rotation the artery is found to be morphologically on the right side. An unusual case in which the left half of the peri-intestinal arterial ring persisted, so that the vitelline artery crossed the intestine on the left, was found in an embryo of 42 mm. This shows conclusively that the artery may persist on either side of the intestine without modifying the direction of the intestinal rotation, and the same is true of the vitelline vein, which in an abnormal embryo modelled by Begg crossed the duodenum on the right. Usually the vein persists on the left side of the tube and the artery on the right side, but these are not essential factors in directing the intestinal rotation.

The vitelline artery, as found by Dexter in the cat ('00) anchors the ileum to the umbilical hernial sac so that the jejunum re-enters the abdomen first and the ileum last of all, stretching the artery to a filamentous strand. This order of return has been confirmed by Broman for the seal, and is probably applicable to man. After the arterial strand ruptures (which usually takes place in embryos somewhat older than those studied, though the strand may exceptionally persist until birth, and rarely through adult life) the place of its at-

tachment to the mesentery may be indicated by an appendage of the mesentery, which Broman finds invariably in seals and names the appendix meso-ilei. According to Fitz, who made an important clinical study of omphalo-mesenteric remains in man ('84), Ruge described such appendages as frequent, but Fitz admits that he himself sought for them with only indifferent success. The models of the 42- and 44-mm. embryos indicate the position, well on the side of the mesentery, where such appendages may be expected, and it seems quite probable that they have been frequently overlooked.

48. *The notochord of an East Indian scorpion.* (Lantern.) WILLIAM PATTEN. Dartmouth College.

In a large scorpion, *Heterometrus* sp.?, recently obtained in Java there is an unusually well developed notochord which presents important new characters of general interest.

A. Embryology. The details of its development are too intricate to be considered here, owing to the presence in it of several different cellular constituents, and owing to its diverse local relations to the spinal cord, to the brain, to their neurilemma sheaths, and to the vascular system.

The main point in its development is that it arises, in close association with the medullary plate, as a median axial cord extending forward from the cephalic end of the 'primitive streak.'

In this important respect it agrees with the notochord of vertebrates and differs fundamentally from the so called notochord of *Balanoglossus* and of *Cephalodiscus*. This fact, when its significance is fully appreciated, should effectively dispose of some of the false ideas that have been entertained, since the earliest days of embryology, concerning the 'gastrula,' the 'blastophore,' and the 'archenteron' of vertebrates, and especially of the idea that the notochord is in some way derived from an outgrowth of the alimentary canal.

B. Structure in the adult. General relations. The notochord of *Heterometrus* is a cylindrical tube, much larger than the spinal cord, extending more than one third the length of the body, that is, from near the root of the tail to the posterior part of the head, or cephalothorax. It lies on the haemal side of the nerve cord, between it and the alimentary canal. The notochord is firmly attached to the nerve cord, its peripheral cells being continuous with those that constitute the neurilemma, and its thick walls, which are relatively firm and elastic, serve to keep the more delicate nerve cord straight and to hold it in place.

Relations to the primordial cranium. In the embryo, the notochord tissue extends as far forward as the forebrain, ending just behind the stomodaeal (infundibular) infolding. The more cephalic portion, however, atrophies, so that, in the adult, the cylindrical notochord appears to terminate abruptly in a point in the hind brain region, although it is continued forward for some distance as a minute, ill defined filament. The cephalic end of the definite notochord lies on

the floor of the endocranium attached to the basilar plate, just inside the occipital ring. In this respect it resembles the anterior end of the notochord in the Cyclostomes.

Relations to the vascular system. - The notochord of *Heterometrus* contains a blood sinus that opens at several points into the vascular system. One opening, at the posterior end, leads into a caudal artery, the others lead into vertical vessels that pass through the floor of the nerve cord, between the longitudinal connectives.

C. Minute structure. The principal part of the notochord is a thick wall of lymphoid tissue containing numerous minute, deeply stained nuclei imbedded in a sharply defined reticulum. Here and there are a few, much larger nuclei, of a very different character, completely filled with very fine chromatin granules.

An ill defined layer of cells forms an outer wall continuous, more especially in the early stages, with the neurilemma of the nerve cords.

A delicate endothelial layer, usually well separated from the notochord tissue, lines the central cavity.

Summary. The available evidence now indicates that the notochord does not make its first appearance in the vertebrates. It first appears in the arthropods, where it occurs sporadically in widely different groups, under widely different structural disguises, and in widely different degrees of development.

While it is to be regarded primarily as a continuous axial organ it may in the same animal be unequally developed in different regions of the body, or present various segmental modifications opposite the ganglionic and interganglionic regions of the nerve cord.

In the vertebrates, the nerve cord becomes more widely established and more uniform in character; that is, it assumes a more constant degree of development throughout the whole class, it extends over a larger part of the body in a given animal, and it is more uniform in character from its anterior to its posterior end.

It will be recalled that the scorpions are the modern survivors of the giant marine Curypterids of the Cambrian and Silurian periods, and that the Curypterids are to be regarded as the probable ancestors of the ostracoderms, and through them of the true vertebrates.

The presence of a well developed notochord in one of the largest of living scorpions is, therefore, a fact of special significance, the more so in view of the facts that in its anatomical structure and in its morphological relations to the alimentary canal, to the nerve cord, and to the cartilagenous cranium, it is in fundamental agreement with the notochord of vertebrates.

Although we have a considerable knowledge of the structure and development of the notochord of arthropods, we have no definite knowledge of its underlying, or primary, function. It is, however, clear in view of its diversified structure in different regions of the same animal and its unequal specialization in different groups of arthropods, that it cannot everywhere have the same function.

In the arthropods, two different functions appear in special cases to be exercised. In some cases the notochord serves as a skeleton, or as supporting tissue; in others it has some obscure relation to the vascular system; or both functions may be exercised, at the same time, in the same animal.

49. *The pathological development of a young human embryo.* C. W. M. POYNTER, Anatomy Department, University of Nebraska Medical College, Omaha.

Doctor Mall has classified pathological ova in four groups as; 1, vesicular forms; 2, ova with neither amnion nor embryo; 3, ova with amnion but no embryo; 4, embryo present but showing more or less degeneration.

This ovum belongs to the fourth group. The chorion, which measures 10 by 8 by 6 mm., was not ruptured; it was mounted in paraffine and sectioned entire.

The embryo is nearly 2 mm. in length, but on account of the unusual development this cannot be taken as an index of age. Estimating from the menstrual history the embryo is about five weeks old.

If atypical environmental factors are responsible for the defective development these factors were probably of a mechanical character, for the chorion seems to be in all respects normal.

The shape of the embryo suggests a development of about 7 somites (Dandy). The neural tube is entirely closed but the differentiation of the brain and cord resemble that of a 3.2 mm. embryo figured by His ('04), except that there is no cephalic flexure.

The body stalk is very much enlarged and represents an outgrowth on the dorsal surface, which is attached to the caudal end of the embryo and extends upward into the amniotic cavity for a distance equal to almost half the length of the embryo.

The circulatory system presents some interesting irregularities. The heart is very much enlarged, extending forward farther than the head, it is roughly 'U' shaped. Only one aortic arch is present on each side and the dorsal aortae are separated throughout their entire length. The right cardinal veins are enormously dilated and there is free communication between the arteries and the veins in the body stalk.

The histological picture is somewhat changed through disassociation (Mall) and infiltration of the tissues with red blood cells.

The embryo furnishes a valuable example of early pathological changes, and in conjunction with a complete clinical history of the pregnancy suggests injury to a normal embryo in the third week of development followed by pathological development for a period for two weeks before death occurred.

50. *Some observations on the ossification of the bones of the hand.* (Lantern). J. W. PRYOR, State University of Kentucky, Lexington.

It is my purpose to call your attention briefly to some of the following observations:

1. The process of ossification is inaugurated much sooner than hitherto supposed.

2. The bones of the female ossify in advance of the male. This is measured at first by days, then months, then years.

3. The chronological order in which the bones of the carpus are ossified is different from that formerly supposed.

4. The bones of the first child, as a rule, ossify sooner than those of subsequent children.

5. Regardless of the variations (normal) the ossification is bilaterally symmetrical.

6. The union of the epiphyses with the shaft takes place much sooner than formerly supposed.

7. Variation in the ossification of bones is a heritable trait.

51. *On the use of the word 'sympathetic' in anatomical and physiological nomenclature.* S. W. RANSON. Northwestern University Medical School.

Confusion and disorder exist in the literature on visceral innervation because of the lack of a satisfactory and comprehensive terminology which meets the needs both of the anatomist and physiologist. The most important contributions to this difficult subject have been made by Langley, who is also responsible for much of the confusion through the unfortunate use of the term sympathetic. Defined in accordance with the accepted anatomical nomenclature, the sympathetic nervous system is that aggregation of plexuses, nerves and ganglia especially concerned in the innervation of the viscera, glands and smooth muscle. Defined in accordance with physiological usage, the term sympathetic nervous system includes only the visceral efferent fibers of the white rami and their postganglionic connections. The terms used by English and German physiologists are given in parallel columns in table 1. Note the divergent use of the word autonomic.

The choice of the adjective sympathetic is unfortunate in any case having in its favor only the advantage of established usage. It is doubly unfortunate that the term should be employed in two such different senses. If it were possible it would be desirable to drop the word entirely and substitute others in its place. As a first step towards adopting a satisfactory terminology it is necessary to have clearly before us the correct interrelation of the parts to be named as well as a statement of what terms may rightly be regarded as synonymous. These relations are expressed in table 2. In the first column the word sympathetic is retained but with the use of the qualifying terms major and minor. In the second column that word is eliminated with the introduction of one new term "the plexiform nervous system." The third column is the same as the second except that the official term "systema nervorum sympatheticum" is substituted for the "plexiform nervous system."

The major sympathetic system is that aggregation of plexuses, nerves and ganglia especially concerned in the innervation of the vis-

cera, glands and smooth muscle. It is obvious that some term is needed to designate this anatomical complex. While established usage may make it necessary to retain the word sympathetic in this connection, it would be desirable to have a term drawn from some obvious gross characteristic of the system. This would suggest the use of the word plexiform. In any case the name chosen is not of so much importance if we understand that it applies to a gross anatomical complex and carries no implication concerning internal structure and function. The word autonomic cannot properly be used in this connection because it refers to a purely efferent system, while all agree that the complex under discussion contains afferent fibers on their way from the cerebrospinal ganglia to the viscera.

It is therefore necessary and proper to have under the major sympathetic or plexiform nervous system a subdivision including the visceral afferent components. Although the only afferent elements that have been satisfactorily demonstrated are fibers having their cells of origin in the cerebrospinal ganglia, this subdivision would give a place for any other sensory elements that might later be shown to exist.

The autonomic nervous system according to English usage includes all general visceral efferent elements both pre and post ganglionic. It is obvious that the term autonomic cannot be used as an equivalent for major sympathetic or plexiform, because it excludes the afferent fibers and because the autonomic fibers extend beyond the plexiform system through the cerebrospinal nerves into the cerebrospinal axis. The autonomic nervous system includes for instance the cells of the intermediolateral group of the spinal cord and the visceral efferent fibers in the ventral roots. It designates a functional group of neurones which are partly within and partly without the cerebrospinal nervous system. It is therefore correct to say that the major sympathetic or plexiform nervous system contains autonomic components though it does not contain all of the autonomic system. The preganglionic autonomic fibers leave the cerebrospinal axis in three streams: the cranial, thoracolumbar and sacral. For many reasons, anatomical, physiological and pharmacological, it is desirable to group the cranial and sacral streams together and contrast them with the thoracolumbar.

The thoracolumbar autonomies include the visceral efferent fibers of the white rami and their postganglionic connections. This group Langley has called the sympathetic nervous system and we suggest that if this word is to be retained at all the designation be the minor sympathetic nervous system.

The other group of preganglionic visceral efferent fibers make their exit along the III, VII, IX, X and XI cranial and the II, III and IV sacral nerves. These with their postganglionic connections constitute the craniosacral autonomies or the parasympathetic nervous system.

In addition to the afferent and efferent components connecting the viscera with the cerebrospinal axis, there is probably present in the

gastrointestinal tract a mechanism for local reflexes. Such local reflexes, if they exist, must depend upon a mechanism different from that of the autonomic system. In order to give a place in our classification for these myenteric reflex arcs we have added a third subdivision for which we have adopted Langley's designation—the enteric nervous system. This should be understood to include only those elements in the gastroenteric plexuses which are involved in the myenteric reflex.

While it may be necessary to retain the word sympathetic in some anatomical names, it is desirable to restrict its use as far as possible. Such terms as sympathetic ganglion or sympathetic fiber should never

TABLE 1
English and German nomenclature contrasted

AUTONOMIC NERVOUS SYSTEM	DAS VEGETATIVE NERVENSYSTEM
Parasympathetic system	Das autonome system
Sympathetic system	Das sympathische system

TABLE 2

Showing the correct relations of the various parts of the peripheral nervous system primarily concerned with visceral innervation giving synonymous terms in parallel columns

MAJOR SYMPATHETIC SYSTEM	PLEXIFORM NERVOUS SYSTEM	SYSTEMA NERVORUM SYMPATHETICUM
Visceral afferent components	Visceral afferent components	Visceral afferent components
Autonomic components ¹	Autonomic components ¹	Autonomic components ¹
Minor sympathetic components	Thoracolumbar components	Thoracolumbar components
Parasympathetic components	Craniosacral components	Craniosacral components
Enteric components	Enteric components	Enteric components

¹ The autonomic nervous system and its two major divisions, the thoracolumbar or minor sympathetic system and the craniosacral or parasympathetic system, include cells in the brain and spinal cord and fibers in the cerebrospinal nerves and cannot therefore be classed under the major sympathetic or plexiform nervous system. Yet the latter is made up in large part of components belonging to one or the other divisions of the autonomic system.

be used. Since the ganglionated cord is associated only with the thoracolumbar autonomies or minor sympathetic system we may with good reason retain for it the name—sympathetic trunk. When it is necessary to designate the ganglia of this system by a general term they should be called autonomic ganglia, since they are all essentially relay stations in the autonomic system. In the names of the individual ganglia, plexuses and nerves no qualifying adjective is needed, as for example, the superior cervical ganglion, the carotid plexus and the carotid nerve. Since the Latin name, systema nervorum sympatheticum has never been used in the same loose way as its English equiva-

lent it might be used without danger of confusion if the Latin form were always retained and the English equivalent never used, even to designate the thoracicolumbar autonomies. All things considered the third column of table 2 seems to offer the best solution of the problem, although the synonyms suggested in the other columns may occasionally be useful.

More important than the selection of satisfactory terms is the recognition of the correct relationship of the various parts to be named. It is clear that there is a gross anatomical complex, the *systema nervorum sympatheticum* or plexiform nervous system. It is also clear that this complex contains autonomic components but not the entire autonomic system and that it also contains visceral afferent fibers. The clear recognition by both anatomists and physiologists of these apparently self evident facts would make possible the adoption of a consistent and uniform nomenclature.

52. *Studies in elastic tissue. III. The behavior of the elastica in arteries following ligation and in the organization of thrombi which ensue.* (Lantern). J. PARSONS SCHAEFFER, The Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia.

In earlier communications of this series of studies an account of the behavior of elastica in the occlusion and the obliteration as such of the ductus arteriosus (Botalli) in man and pig was presented. In both instances elastica was found to play an important rôle in the occlusion of the lumen of the postfetal ductus. Many new elastic fibrils were demonstrated; some, doubtless, the product of preformed elastica, others apparently the product of protoplasmic activity of cells in loco and of certain wandering cells which found their way into the occluding mass.

It is a well known fact that elastic tissue behaves diversely in the various pathologic states. Even in the organization of thrombi elastic tissue presents bizarre appearances which seem dependent upon the etiological factor back of the thrombus. In view of the latter, an investigation of the behavior of elastic tissue in arteries after ligation was undertaken. Opportunity was also afforded in this study for observing the behavior of elastic tissue in the organization of thrombi following ligation. This work is merely in a preliminary state and is briefly abstracted herewith.

The rabbit was the animal used for the experimental work. Twenty-five animals were operated upon after the usual technic preparatory to surgical procedures and anaesthetization with ether. Twenty-four animals recovered rapidly from the operation with no apparent ill effects. One animal died of an infected wound.

The common carotid artery of either the right or the left side was secured and ligated at two points, an inch or less of artery intervening between the two ligatures. The distal ligature was usually placed first, thus insuring a blood-filled segment between the ligatures. The ligatures were placed variously: In some instances the endothelial

walls were brought into mere apposition without injury, in others the ligatures were tightly placed with obvious endothelial injury. In most instances the blood was left in the artery segment, in others a slit was cut and the blood was allowed to escape. Again the artery segment was considerably traumatized, in others handled with a minimum trauma. At selected periods after the operation the animals were again etherized and the ligated vessels secured for study.

It is a well known fact that clots may form within blood vessels upon the introduction of foreign material. Injured endothelial cells may act as a foreign substance. In the present series it was found that in those cases in which the artery was ligated gently, i.e., the endothelial walls brought into mere apposition, the blood between the ligatures remained fluid for a relatively long time. However, when the artery was considerably traumatized and the ligatures tightly placed so as to injure the endothelium, intravascular clotting between the ligatures ensued much sooner. Again, the blood became thrombosed in one portion of the artery segment between the ligatures and at another it would remain fluid much longer. Indeed, at some points the blood elements would undergo disintegration and no thrombosis or organization occur in that position. The subendothelial stratum would thicken instead and gradually encroach upon the lumen. Later the cone of the thrombus from another portion of the segment of the artery would grow into the narrowed lumen.

After an interval of from five to twelve days following the ligation, there was found here and there a positive cellular thickening of the subendothelial stratum. It is well to recall that in the common carotid artery of the rabbit, the endothelium at many places rests directly on the inner elastic lamina. At other places a few scattered connective tissue cells intervene. In other words, the subendothelial stratum is for the most part wanting. It seems scarcely possible that the cells in the thickened subendothelial stratum all have their source from the few connective tissue cells in loco. Furthermore, the endothelium is for a considerable time intact and continuous. This is especially so in those cases in which the intravascular clotting is delayed. It is not believed, therefore, that the endothelium contributes to this initial cellular thickening. There is another possible source, viz: the connective tissue cells of the media and adventitia. It is probable that some of these cells wander into the subendothelial stratum and contribute to the early cellular thickening. Indeed, serial sections reveal occasionally connective tissue cells on their way through the inner elastic lamina and at points one can demonstrate a number of cells near the inner elastic lamina looking vertically towards the lumen of the vessel. Of course, in those cases in which the blood early becomes thrombosed and organization sets in, the endothelial lining soon breaks up and undergoes proliferation and doubtless aids in the organization of the thrombus. In such instances the endothelial cells become modified and seem to assume a fibroblastic rôle.

When the blood remains fluid for a long time, the subendothelial stratum becomes thicker and thicker at the expense of the lumen. The enclosed blood is encroached upon. Usually the cone of a growing thrombus from another portion of the artery segment, pushes its way into the constricted lumen. In cross-section the cone of the thrombus is entirely free. Serial sections show, however, it to be a part of a thrombus from another portion of the artery segment undergoing organization.

It is the very early but obvious thickening of the subendothelial stratum that is of prime interest here. Careful study of thin sections reveals in the outlying portion of the exoplasm of the cells in the thickened endothelial stratum very delicate, granular-appearing elastic fibrils. These fibrils in a sense immesh cells, and their appearance, position, and relation indicate that they are the product of protoplasmic activity. At this time the inner elastic lamina appears normal and healthy. The delicate, granular elastic fibrils have no connection with it.

Subsequently the inner elastic lamina, shows here and there a splitting into several layers. In these positions the lamina becomes fragmented, the fragments projecting into the subendothelial stratum and seemingly undergo proliferation in this position. The inner elastic lamina in such positions appears 'moth-eaten.'

The thickened subendothelial stratum is, therefore, made up of connective tissue cells and collagenous and elastic fibrils, the latter of a dual source. When the blood remains fluid for a long time the subendothelial stratum will continue to thicken by a multiplication of these elements and at the expense of the lumen. It reminds one of an obliterating arterio-sclerotic process.

If intravascular clotting early ensues as is often the case the subendothelial stratum has little time to thicken. The endothelial lining is broken up. There is a rapid migration, especially along fibrin bridges, of the elements of the subendothelial stratum and of endothelial cells into the thrombus to aid in its organization. It appears that the endothelial cells assume a fibroblastic rôle in the rapid organization of the thrombus.

The newly formed elastic tissue extends ring-like into the organizing thrombus, forming a net-like mass. This gradually pervades the whole thrombus. Newly formed blood vessels within the thrombus always have very definite inner elastic lamina.

In presenile gangrene, the result of a thrombo-angiitis obliterans probably due to an infection, there is always an absence of elastic fibers save a few around the larger canalizing vessels. Here we have a definite and positive organization of a thrombus with little participation of the elastica. In an arterio-sclerotic process (without thrombosis) in which the lumen of the vessel may become obliterated, we have an abundance of elastic fibers aiding in the occlusion. In the present study where thrombi were experimentally produced under aseptic conditions, elastic tissue plays an important rôle in thrombus organization.

In a few instances a longitudinal slit was cut into the artery segment between the ligatures and the blood allowed to escape. Where the separation of the cut edges was not too great, the elastica aided in the repair. New elastic fibrils were formed from the cut ends of the circular fibers of the inner elastic lamina, in an effort to establish continuity of structure of elastic fibrils. However, where the separation of the cut edges was fairly great, the lumen of the vessel was rapidly encroached upon by endothelium and connective tissue elements. New elastica aided in the occlusion of the lumen, but the cut ends of the circular fibers of the inner elastic lamina did not throw out new fibrils to the same extent as in the positions where the cut edges of the vessels were separated to a less degree. Seemingly when the cut ends of the circular fibers of the inner elastic lamina are less separated, some stimulus is forthcoming to bring about proliferation of the cut ends in an attempt to establish continuity of structure of elastic fibrils. Where the separation is too great, the cut ends of the circular fibers of the inner elastic lamina not only fail to give rise to new fibrils, but they actually seem to undergo disintegration. A study in the larger series is, of course, necessary to draw conclusions. The behavior of the longitudinal fibers of the inner elastic lamina were not studied.

*53. *The early stages of the development of the great veins and of the hepatic circulation in the cat.* H. VON W. SCHULTE, Anatomical Laboratory of Columbia University.

That the duct of Cuvier has a more complicated history than is expressed in the familiar statement that it serves to connect the pre- and post-cardinal veins with the sinus venosus, has long been known to embryologists. A model of the great veins of a cat embryo of 5 mm. by Dr. Huntington, shows the participation in its formation of a plurality of elements. Of this it has been stated that "the duct of Cuvier is formed through the confluence of the precardinal, the postcardinal, the omphalo-mesenteric and the umbilical veins." (Huntington and McClure: *Am. Jour. Anat.* vol. 10, 1910, p. 231 and fig. 25). It is now possible, through the opportunity of suitable material, to record the stages antecedent to the condition above described. In embryos having 12 to 14 pairs of mesodermic somites the umbilical vein extends to the anterior limb bud and receives in addition to plexiform tributaries from the somatopleure, the precardinal vein. This primitive drainage of the precardinal has previously been described by His and by Lewis (*Proc. Ass. Am. Anat.*, 17th Sess., 1903). In the cat at 14 somites the umbilical crosses the omphalo-mesenteric vein dorsally and here opposite the interval between the third and fourth, somites anastomoses with the latter (*Mem. Wistar Inst.*, No. 3, fig. 16).

Subsequently with the lengthening of the foregut and the development of a ventral body wall, the omphalo-mesenteric vein for a segment of its course becomes parallel to the umbilical which has been carried ventrad and now lies against the lateral side of the omphalo-mesenteric. As the umbilical extends to the limb-bud a considerable

segment of it extends cephalad of its communication with the omphalo-mesenteric vein. With this segment the precardinal retains its connection, a portion of the umbilical extending beyond the junction. Additional taps are established between the parallel segments of the umbilical and omphalo-mesenteric veins and a portion of the umbilical is gradually incorporated into the latter vessel. This is accomplished in embryos of 4 to 5 mm. The umbilical now appears as a tributary of the omphalo-mesenteric vein and its cephalic segment gradually falls in line with and forms the continuation of the precardinal. Meanwhile the postcardinal has increased in size and with the resolution of its plexiform connections with the umbilical establishes a secondary anastomosis with the precardinal. The blood from both cardinal veins from this period is carried to the heart through the proximal segment of the precardinal, a portion of the umbilical and finally a portion of the omphalo-mesenteric, the resulting vessel constituting the duct of Cuvier.

The earliest capillaries of the septum transversum make their appearance in close proximity to the entoderm of the foregut in the interval between the omphalo-mesenteric veins and immediately caudal to the sinus venosus. Angiogenesis is active and prior to the appearance of the hepatic diverticulum a plexus is formed which becomes connected at the side with the omphalo-mesenteric veins and cephalad with the sinus venosus. These capillaries are preceded by the formation of angiocysts among which are scattered small blood islands. Many of the vessels are of considerable size with wide irregular dilatations. Subsequently this plexus becomes connected with that about the lung bud and is evidently an accelerated part of the general peri-oesophageal or splanchnic plexus (Davis, Brown).

The hepatic diverticulum is present in embryos of 19 and 20 pairs of mesodermic somites, and naturally is in immediate contact with this plexus of the septum transversum. The ventro-lateral sprouts of the liver invade the septum transversum which increases rapidly in size and continues to be actively angiogenetic. An invasion of the omphalo-mesenteric and umbilical veins is not present until the stage of 4 to 4.5 mm. At this period on the right side a mass of liver sprouts fills the angle of confluence of these vessels and a long falciform process of liver grows out upon the dorsum of their fused segment. Here upon follows rapidly the resolution of this segment into sinusoids and a reduction in the size of the distal segment of both veins. The left omphalo-mesenteric has increased greatly in size, the left umbilical moderately. In the ventral region of the liver the capillaries are abundant and continue to be connected with both omphalo-mesenteric veins and with the sinus venosus. Many of them are separated from immediate contact with the liver sprouts by the interposition of a moderate amount of young connective tissue. This topography and their antecedence in time of the hepatic sprouts, substantiates Mollier's statement of the independent formation of capillaries in the septum transversum and indicates that a considerable portion of the hepatic

vessels are formed before and independently of the resolution of the omphalo-mesenteric and umbilical veins into sinusoids.

*54. *Observations on the osteology of the porcupine fish.* (*Diodon hystrix*).

R. W. SHUFELDT, Washington, D. C.

Several years ago I made disarticulated skeletons of *Diodon hystrix*, and compared them with others I had prepared of the Burr fishes (*Chilomycterus schoepi*); a skull of some species of *Ovoides*; the skeletons of several species of *Spheroides*, popularly known as Swell fishes, and of two or three of the trunk fishes, as *Lactophys triqueter*, *L. tricornis* and others. Also with the Butterfly-fishes (*Chaetodon*) and Angel-fishes (*Angelichthys*), and, finally, with several species of the File fishes of the family *Monacanthidae*, including *Monacanthus hispidus* and two or three species of *Alutera*, the last kindly presented me by Dr. Francis E. Sumner, of Woods Hole, Massachusetts.

This material is before me at the present writing, as well as a series of photographs I have made of not a few of these skeletons.

Jordan and Evermann, in the second part of their most useful work on the "Fishes of North and Middle America," place all of the above-named forms in groups more or less nearly allied to each other. For example, in the Suborder *Squamipinnes* (or the Scaly-fins) we find the *Chaetodontidae*, the species of the genus *Angelichthys* (Angel-fishes), and various others, as the Doctor-fishes, which I have likewise osteologically examined (*Teuthis*). Following the *Squamipinnes*, we have the Suborder *Sclerodermi*, in which we have the File-fishes (*Monacanthidae*); the Suborder *Ostracodermi*, created to contain the Trunk-fishes, and, finally, the Suborder *Gymnodotes*, which includes the families *Tetraodontidae* or the Puffers, the family *Canthigasteridae* or Sharp-nosed Puffers, and, lastly, the family *Diodontidae* or Porcupine fishes, in which group we find the subject of my researches of which the present abstract is a brief résumé.

All the *Diodontidae* are very sluggish fish, and they usually remain near the bottom, slowly swimming about amidst the marine vegetation and the various kinds of corals there found. Their armor-spines are far more formidable than we find them in their relative, the Puffers, and the bones entering into the formation of the mandibles unite so completely in the adult fish, that the sutures almost, if not entirely disappear. This has led some ichthyologists to believe that each jaw is composed of a single bone.

This remarkable fish, with its curious coat-of-mail bearing from head to tail the long, bony, and very sharp spines, may grow to be a yard long, or even slightly longer in some specimens which have been met with off the Bermudas. It is very abundant everywhere in tropical seas, especially in the Hawaiian Islands, coast of Lower California, and from the Carolinas all the way around the eastern coast of North America. It is useless as a food fish, while its odd-looking, spiny skin is frequently found in various places and in collections, blown up and dried as a curiosity; mounted specimens also are preserved in

a similar manner. There are those who believe that the flesh of the Porcupine fish is poisonous if eaten; but there is absolutely no truth in this idea.

Many are familiar with the habit of the Puffers or Swell-fish of blowing themselves up when caught and taken out of the water. A slight scratching of the abdomen will induce the fish to do this. In *Diodon* this capacity of inflation is very much feebler, though the fish has the power of gulping in sufficient air to cause it to float, belly upwards, on the surface of the water. Many years ago, I saw one floating in this manner in the Florida Straits, when the surface of the water was smooth and at rest.

Apart from the dense and massive mandibular bones and a few of the smaller bones of the cranium, the entire skeleton of *Diodon*, as in the case of its near allies, differs from that of most true teleosts, in that the bones composing it are quite elementary in appearance and texture—as though they were composed of a whitish paper pulp rather than of true bone. This does not apply, however, to the spines of its coat-of-armor; they are especially hard and dense, and as strong as steel. When my final paper on the osteology of this fish is published, there will appear in it an account of the most interesting articulations among these spines, which are imbedded in the integuments so that the fish can elevate or depress them at will. After the heavy and massive jaws have been removed, the cranium is seen to be of a sub-cubical form, being almost as broad and as deep on its anterior facial aspect as it is upon its dorsal superficies.

The superior mandible is, as just stated above, a very heavy and massive bone, being composed of the thoroughly fused maxillaries and pre-maxillaries, the sutures between the two being plainly visible, but only faintly in the median line between the premaxillaries. As one bone, it has the form of a broad U, and the corrugated dental plates are similar in the two jaws. On the superior edge of either maxillary there is a free admaxillary.

Viewing the cranium from in front, it is to be noted that it is the nasals that have, in chief, been modified to articulate with the upper jaw; and from them, upon either hand and separated by a wide interval, descends a long column of bone to include the mandibular articulation below. In either one of these columns we have an extraordinary arrangement of the quadrates and entopterygoids.

This is difficult to make clear without the use of a figure of the skull; but such a figure and others have already been made, and will illustrate my final contribution to this subject.

On dorsal aspect, the skull of the Porcupine fish is quadrilateral in outline, the anterior moiety being formed by the large and spreading frontals. All the bones on the roof of the cranium are firmly united together through a peculiar overlapping articulation. Most of the sutures can be made out; while, in the case of others, the boundaries have nearly disappeared in the adult.

There is a small cranial capacity, and the cranial casket is entirely open anteriorly; while anterior to its inferior border the presphenoid passes forwards as a trumpet-shaped bone, with the small end attached to the basisphenoid.

On either side of the skull a rather large symplectic is present, making its usual articulations with the surrounding bones.

The remaining bones of this part of the skull will be fully described later on in my formal monograph on the osteology of this truly remarkable fish.

As in the case of the upper jaw, the mandible is a very massive, U-shaped structure, supporting a row of small teeth, and a somewhat removed, medium, subelliptical plate of others, quite similar to the dental armature in the upper jaw. The articulation with the quadrate is quite extensive.

The bones of the branchial apparatus and tongue are all handsomely developed, but cannot be well described here without the use of figures.

Passing to the spinal column, we find the vertebrae large and unusually well developed for a fish having a coat-of-mail. In the Burr fishes for example, this is not the case, and they possess a very highly developed armor (*Chilomycterus*). *Diodon* has the leading eight (8) vertebrae almost devoid of prominent lateral and inferior apophyses. In the ninth vertebrae, however, these suddenly appear, to become broad and spreading in the 12 to 15 vertebrae. There are 20 vertebrae all told in the spine of this fish, and they present some very remarkable characters. The terminal one is much compressed laterally, and very large. It supports, upon either side, a prominent hypural spine, and offers a long shape margin for articulation with the caudal fin, or peduncle. The neural spines supporting the dorsal fin fuse below with the neurapophyses of the 12 to 16 vertebrae, which have their neural spines powerfully compressed laterally, and otherwise modified to receive them. The rays of all the fins are well ossified, and the actinosts thoroughly developed, in some instances of considerable size. The important comparisons of the skeleton of *Diodon* with those of its near relatives will appear in my formal contribution, as they are of too extensive a nature to be incorporated in the present abstract.

*55. *The ovarian cycle in mice.* H. P. SMITH, Anatomical Laboratory, University of California.

During the last year a study of the ovulation cycle in mice has been undertaken in conjunction with Dr. J. A. Long, a preliminary report of which has been published elsewhere. On that occasion we presented the results derived from the study of serial sections through the oviducts and ovaries of 61 mice which had been isolated from their litters and from males immediately upon parturition, and killed at varying intervals thereafter. In this way we avoided any possible effect of lactation or sexual excitement due to the presence of the male. That study, which has been carried out over a period of 91 days fol-

SERIES	LENGTH OF TIME BETWEEN PARTURITION AND KILLING	POSITION OF EGGS IN OVIDUCT	CALCULATED LENGTH OF TIME BETWEEN PARTU- RITION AND OVULATION
121	20 hours	Ovarian one-third	20 hours
186	33 hours	One-third way down	20 hours
216	40 hours	Nearly one-half way down	20 hours
488	48 hours	In second fifth	20 hours
448	48 hours	In ovarian fifth	20 hours
489	60 hours	Two-fifths way down	20 hours
483	72 hours	In last fold	20 hours
490	96 hours	At entrance to uterus	20 hours
491	96 hours	At entrance to uterus	20 hours
414	96 hours	Near entrance to uterus	20 hours
492	108 hours	In last fold	20 hours
494	6 days		
415	6 days		
460	7 days		
416	8 days		
485	8½ days		
486	9 days	In loop leading to last fold	6½ days
463	9 days		
417	10 days		
418	10½ days		
419	11 days		
420	12 days		
450	12 days		
496	12½ days		
421	14 days		
497	15 days		
422	16 days		
498	17½ days		
462	18 days		
423	18 days	Uterine one-third	16½ days
447	18½ days		
432	18½ days		
459	18½ days	Uterine one-fourth	16½ days
484	18¾ days	One-fifth way down	18 days
461	19 days		
424	19 days	Ovarian one-fourth	18 days
434	19 days		
500	19 days		
501	19 days	One-fourth way down	18 days
502	19 days	One-fifth way down	18½ days
487	19½ days	At ovarian end	19 days
504	19½ days		
439	19½ days		
505	19¾ days		
506	20 days		
425	20 days	Little over half way down	18¾ days
507	20½ days		
426	20½ days	Uterine one-fourth	18½ days
509	21 days		
427	21¼ days		
465	21¾ days		
428	22 days		

lowing parturition, pointed to the conclusion that ovulation in mice occurs spontaneously at intervals of about $17\frac{1}{2}$ days.

The present report of continued work undertaken at the suggestion of Dr. Long, represents an attempt to examine a greater series of cases during the first 22 days post partum in order to detect variations which might occur as regards the time of occurrence of ovulation.

Fifty-two cases are tabulated on p. 92. It will be seen that it is possible to state that the next spontaneous ovulation following upon the one which so quickly succeeds parturition occurs between $16\frac{1}{2}$ and 19 days after parturition. The average of the nine such cases observed in this study is 18 days after parturition or in other words a few hours less than 17 days after the ovulation following littering.

A surprising result of the above table must now be alluded to, i. e.: the spontaneous ovulation in question occurred in only 42 per cent of the cases in which it would be expected (9 cases in 21 cases). No explanation is offered for this irregularity.¹ Possibility of individual cases of still greater variation is indicated by one instance in which a second spontaneous ovulation occurred at $6\frac{1}{2}$ days post partum (case 486).

Furthermore, the early part of the present series gives us a good indication of the rate of migration and time of survival of unfertilized eggs in the oviduct of the mouse. A part of the above table may be presented again in the form given below. From it may be inferred that the ovum of the mouse consumes approximately two days in

SERIES	LENGTH OF TIME BETWEEN ESTIMATED OVULATION (TIME OF PARTURITION PLUS 20 HOURS—LONG AND MARK ²) AND KILL- ING	POSITION OF EGGS IN OVIDUCT
	<i>hours</i>	
121	0	Ovarian third
186	13	One-third way down
216	20	Nearly half way down
488	28	In second fifth
448	28	In ovarian fifth
489	40	Two-fifths way down
483	52	In last fold
490	76	At entrance to uterus
491	76	At entrance to uterus
414	76	Near entrance to uterus
492	88	In last fold

¹ Since one case of estimated ovulation at 19 days was found it will be well to recognize that other instances of as late ovulation could conceivably be represented by cases 489, 462, 447, and 432, killed prematurely.

² Precisely the time of occurrence of ovulation did not interest Long and Mark, but from their careful table of stages of ovulation observed in 19 mice killed from $14\frac{1}{2}$ to $28\frac{1}{2}$ hours post partum one may conclude that in over 75 per cent of all cases ovulation had occurred by the twentieth hour after parturition. (Long and Mark. The maturation of the egg of the mouse. Publ. Carnegie Institution of Washington No. 142, p. 20, table 4, 1911.)

traversing the greater part of the oviduct but that it also waits in the last uterine loop or portion of the oviduct approximately one day or more so that three days must be allowed for the completion of migration.

It was in fact by the use of the second table that estimations of time of ovulation were recorded in table 1.

56. *The effect of hypophysectomy upon the subsequent growth and development of the frog (Rana boylei.)* P. E. SMITH, University of California, Berkeley.

In the operated specimens the hypophyseal rudiment was removed soon after it had commenced to invaginate, that is shortly after the closure of the medullary tube. Controls consist of specimens upon which the operation was unsuccessfully attempted, and of unoperated animals. All animals were reared under identical conditions.

In the hypophysectomized specimens particular attention is called to the non-development of the hind legs; to the pronounced decrease in the size, parenchyma, and colloid of the thyroid gland; and to the profound reduction of the epidermal pigment.

Only one specimen of the group in which the hypophyseal rudiment was ablated, developed legs at the normal rate. Sections show that, although the glandular portion of the hypophysis was totally extirpated, yet the thyroid developed normally and the epidermal pigment was typically reduced.

*57. *Changes in the relative weights of the various parts, systems and organs of very young albino rats underfed for various periods.* C. A. STEWART, Institute of Anatomy, University of Minnesota.

Forty-three rats have been used, including 20 controls and 23 test animals. Of the controls 6 were dissected at an average net body-weight of 9.9 grams, 4 at 12.9 grams and 10 at 14.7 grams. The test rats were starved for intermittent periods starting a few hours after birth, and were dissected at the age of three weeks (7 rats), six weeks (7 rats), and ten weeks (9 rats), the average net weight at each age being 10.1, 12.5, and 14.9 grams. On account of the normal variability, and the small number of observations, conclusions are somewhat uncertain in some cases.

As to the body proportions, the weights of the head, trunk and extremities are practically normal in the test rats as compared with the (younger) controls of the same weight. The existing small differences may appear more significant upon the addition of further data.

Of the systems, the skeleton and visceral group (as a whole) show a considerable increase in weight in the test rats. There is also a slight increase in the musculature. The integument is variable, showing an increase in the rats underfed three weeks, but a marked decrease in the later periods. The 'remainder' appears to decrease greatly in the rats underfed three weeks, while in the rats underfed six and ten weeks the change is less marked.

Of the individual viscera, the spinal cord, eyeballs, liver, stomach and intestines (empty), suprarenals, kidneys, testes, hypophysis and ovaries show a definite increase in weight in all three groups. The brain and epididymi show a marked increase in the rats underfed the shorter period (3 weeks), but are doubtful later. The heart and spleen are variable, each apparently losing weight during the earlier fasting period (3 weeks), but increasing in weight so as to surpass the controls during the later periods.

The lungs suffer a considerable loss in weight in all three groups and likewise the thymus (especially at 10 weeks).

There is apparently no marked change in the weights of the thyroid and pineal body.

In general the results agree fairly well with those obtained by Jackson (*Jour. Exp. Zool.*, vol. 19, no. 2, '15) in rats held at maintenance for various periods beginning at 3 weeks of age. There are, however, several differences found in the rats of the present experiment, in which the underfeeding was begun shortly after birth. These differences are probably due to the varying tendencies to growth and maintenance among the various organs at this earlier period.

*58. *The existence of a typical oestrous cycle in guinea-pigs and its histology.* CHARLES R. STOCKARD and GEORGE N. PAPANICOLAOU, Cornell University Medical College, New York City.

Normal guinea-pigs of our control stock possess a regular periodic proestrus, occurring every fifteen or sixteen days. This fact was ascertained by examining the vaginae by means of a speculum every day during different seasons of the year. The flow, which marks the proestrus activity, is not very abundant and consists largely of desquamated epithelial cells and some mucous secretion.

In the first stage there is a flow of a mucous fluid filled with superficial squamous cells of the vagina. A few hours later a thick cheese-like substance occurs in the vagina. This consists almost entirely of the deeper epithelial cells which preserve their epithelial structure and often remain together in groups or actual pieces of epithelial tissue. This thick vaginal substance after a few hours becomes more fluid in consistency and pus-like in appearance. A microscopical examination at this time shows a very large amount of polymorphonuclear leukocytes among the epithelial cells and the beginning of an active phagocytosis. The result of this phagocytosis is that in a few hours the vagina is almost completely cleaned and no longer contains the menstrual substance except for a little fluid containing leukocytes and a few broken down epithelial cells. The occurrence of either red blood corpuscles or hemoglobin in the menstrual flow takes place only in the later stages.

The active phagocytosis may probably account for the fact that none or very little of the pus-like fluid ever flows out from the vagina. The leukocytes, migrating from the subepithelial capillaries of the uterus and the vagina, as microscopical sections show, attack the desquamated

epithelial cells within the lumen of the uterus and vagina and there begin to destroy them. The entire process of menstruation is not long, its duration being less than twenty-four hours. But during this time the entire genital tract is inflamed by a very active circulation of blood.

The anœstrous period of the uterus and vagina is characterized by the absence of the secretions and the constant presence of leucocytes. Especially the first week after proœstrum, the vagina is very clean and dry. During the second week and particularly a few days before the next proœstrum there is a little mucous fluid in the vagina, and this contains some leucocytes and a few squamous cells. The massive desquamation and the abundant thick secretion, however, occurs very regularly every fifteenth or sixteenth day.

The menstruation or proœstrum seems to be closely followed by an ovulation. At this stage of our preliminary study of the correlation between menstruation and ovulation observations indicate that the ovulation occurs about eighteen hours after the height of menstruation (the presence of the abundant thick secretion).

*59. *The morphological changes of the idiosome during the spermatogenesis of the guinea-pig.* C. R. STOCKARD and GEORGE N. PAPANICOLAOU, Cornell Medical School, New York City.

La Valette St. George was the first to describe the idiosome under the name 'Nebenkern' in 1865-1867. Since then this body has been described under many different names in a great number of papers treating the processes of spermatogenesis and oögenesis in different animal classes. Meves ('99), described the structure during the spermatogenesis of the guinea-pig and called it the 'idiozom' (from idios—own, and zoma—belt-). Regaud ('10) proposed a modification of this term to idiosome (from idios—own and soma—body). We accept the modification as better fitting our conception of this structure.

Not very much specific study has been devoted to the idiosome probably on account of the fact that its close position in relation to the centrosomes has made some observers fail to realize that it is in independent body, having its own peculiar history. There is certainly at particular stages a very close relation as to position between the idiosome and the centrosomes, but the relation is temporary. In only one stage in the spermatogenesis of the guinea-pig, are the centrosomes really in very close connection with the idiosome, and this is in the primary spermatocytes. During this stage the centrosomes are enclosed in the center of the idiosome. But as soon as the spireme begins to form they come out of the idiosome to play their usual rôle during the nuclear division, while the idiosome continues its independent development with a surprising succession of highly specialized morphological changes. In the later stages, the secondary spermatocytes and spermatids, no connection is to be observed between the centrosomes and the idiosome.

The idiosome in the male germ cells of the guinea-pig is present even in the spermatogonia. At this stage the idiosome is of somewhat irregular shape and appearance and its internal structure is not clear and definite. First in the primary spermatocytes, the idiosome takes a regular spherical form and shows a clear differentiation into two zones, a peripheral, the idioectosome, and a central, the idioendosome. The latter zone is completely enclosed by the former. When the prophase begins in the division of the primary spermatocytes the idioendosome breaks up into a number of granules the idiogranulomes. During the progress of division, the idioectosome also breaks into smaller pieces, which, together with the idiogranulomes are dispersed throughout the protoplasm. In this way a uniform distribution of the idiosomatic material is secured during the division process.

At the end of the division the idiogranulomes and the pieces of the idioectosome begin to flow together near the daughter-nuclei and thus form two daughter-idiosomes. In the secondary spermatocytes the idioectosome takes a regular spherical shape, while the idiogranulomes form a group of closely arranged granules in its center, as if they were preparing to fuse. A fusion does not take place, however, probably on account of the short existence of the secondary spermatocytes.

During the division process of the secondary spermatocytes the idioectosome again breaks up into small pieces to be distributed in the cytoplasm together with the liberated idiogranulomes. In this way repeating the phenomenon which occurred in the primary spermatocytes. In the telophase a new flowing together of the idiogranulomes and of the pieces of the idioectosome takes place near the new daughter-nuclei, forming the idiosomes of the spermatids.

The idiosome of the spermatids thus has the same formation as that of the secondary spermatocytes, consisting of a large idioectosome containing a great number of small idiogranulomes. The idiogranulomes are each enclosed in a small vacuole, the idiogranulotheca. Soon after the spermatids are formed the idiogranulomes and idiogranulothecae fuse together into larger and larger masses and vacuoles until finally they form a single big body, the idiosphaerosome surrounded by a large vacuole the idiosphaerotheca. The idiosphaerosome, as soon as formed, begins to secrete on its surface furthest from the nucleus a new substance, showing a different color reaction and a vacuolar consistency. This substance, the idiocalyptososome, covers in a cap-like fashion the remains of the idiosphaerosome now called the idiocryptosome. At this time the idioectosome forms a cap above the idiosphaerotheca and later on when the secretion of the idiocalyptososome is about complete the idioectosome becomes detached as a separate body. It then moves along the nuclear membrane and finally goes over into the remains of the protoplasm on the posterior pole and is eliminated.

In later stages of development the idiocryptosome comes to lie in the form of a small cap on the anterior pole of the nucleus. The calyptososome grows into a very large body, always showing a vacuolar consistency. In a still later stage, when the spermatid comes into con-

nection with a Sertoli cell, the idiocryptosome and the idioalcalyptrosome are elongated in the form of two cones, the one enclosed by the other. During the final metamorphosis of the spermatid, the cryptosome again assumes the form of a cap, covering the anterior part of the head of the sperm, while the calyptrosome loses its original vacuolar consistency and forms an outer larger cap covering over the inferior cryptosomal cap. The calyptrosome cap and part of the head of the spermatozoön are covered by the idioalcalyptrotheca, which is the fully developed and transformed idiosphaerotheca.

A point of special interest is the appearance of small granules, comparable to the idiogranulomes, in the nucleus of the germ cells during all stages of development. These we have termed caryogranulomes. Such granules are to be seen in the nucleus of the spermatogonia, the primary and secondary spermatocytes and the spermatids. The caryogranulomes are usually of small size about that of the smallest idiogranulomes, but in some stages they also seem to fuse together forming large granules. Their appearance, their color reactions and their tendency to fuse all show great similarity to the idiogranulomes, yet no genetic relation was directly observed. The caryogranulomes persist up to the time of the metamorphosis of the spermatid into the spermatozoön. Then they dissolve in the head of the sperm in the same way as does the chromatic material of the nucleus.

The new points brought out by this study are the following: First, The recognition of the idioendosome. Second, The description of the formation of the idiogranulomes through the breaking up of the idioendosome. Third, The persistence and the behavior of the idiogranulomes during the divisions of the primary and secondary spermatocytes. Fourth, The existence of the caryogranulomes and their development. Fifth, The exact manner of the formation of the calyptrosome and its vacuolar structure. Sixth, Certain peculiarities in the development of the cryptosome. Seventh, The double nature of the idiosome, consisting of an ectosomatic and an endosomatic substance, each having an independent development. Eighth, the rôle of the granulation of the idioendosome in serving to distribute the idioendosomatic substance during each division.

*60. *Some studies on the venom gland and its excretory duct in crotalus horridus.* VIVIAN STRAHM. Department of Anatomy, University of Kansas. (Introduced by J. Sundwall.)

The venom gland has been studied in several species of reptile. It has been reported by Leydig for *Vipera berus* (Schultze's Arch., Bd. 9, pp. 598-652); by Emery for *Naja-haje* (Arch. fur Mik. Anat., Bd. 11, pp. 561-569); and by Holm for *Heloderma suspectum* (Anat. Anz., Bd. 13, pp. 80-85). Dr. Mitchell in his researches on the chemical and physical properties of the venom in *Crotalus* included a description of the venom apparatus (Smithsonian Contributions to Knowledge, vol. 12).

For my studies, heads from two specimens of *Crotalus horridus* were used. The reptiles were killed the latter part of January, after about three months captivity, and during their normal hibernation period.

The reptiles were chloroformed and the heads severed about an inch behind the jaw. At once, the skin over the glands was reflected, one gland from each head removed, and cut into small pieces. Part of these bits were fixed in neutral form-Zenker, the remainder in Bensley's aceticosmic, bichromate mixture. The heads with the remaining glands undisturbed were fixed in acetic Zenker, and run to 70 per cent. The small pieces were used for histological demonstrations; the heads, for gross structures and relationships.

The venom gland, a flat, smooth oval body, tapering at the anterior end to join the excretory duct, lies on the side of the head above the jaw and behind the eye. The posterior border extends 30 or 40 mm. behind the commissure of the lips. The anterior end lies just below and behind the eye. The gland is thus bounded by the skull behind the eye, the anterior and the middle temporal muscles, the external pterygoid muscle and the skin below and in front of the anterior temporal muscle. The gland is covered with a single layer of dense, fibrous connective tissue. This layer, which is continuous with the layer covering the duct, gives rise to three ligamentous bands and attachment to one.

The excretory duct throughout its course is imbedded in the fascia between the skin and the skull. As it leaves the gland, it is pointed forward and downward. In front of the eye it turns upward, rises to the level of the orbit and again turns sharply downward. It passes under the fossa, around the superior maxillary bone and ends at the base of the poison fang.

The outer surface of the duct is of uniform diameter throughout its length except for a distance of 35 or 40 mm., as it crosses the lateral side of the superior maxillary bone. Here it increases slightly in diameter. Dr. Mitchell noted this thickening and believed it was due to the presence of a sphincter muscle. I found a small mucous gland in this region surrounding the duct. The capsule of the duct also forms the capsule of the gland. No smooth muscle fibers occur in this region nor in any other portion of the duct or gland. This observation is not in accord with that of Dr. Mitchell.

Venom gland and excretory duct

The venom gland is made up of wide branching tubules lined with a single layer of columnar epithelium which is supported by a connective tissue framework. These tubules are short and straight. They are directed backward and outward from their openings. The lumina of the tubules are very wide throughout. The number of tubules seen in any cross section increases rapidly on passing from the anterior to the posterior portion of the gland.

In the anterior third of the gland, on the medial side, a wide lumen forms a receptacle which serves to store collecting secretion. It is continuous with the lumen of the excretory duct.

The lumen of the duct is wide, unobstructed and roughly circular through the three flexures. After passing the third flexure, the walls of the duct are crowded and much folded by the enlargement of the mucous gland which appears in this region.

Leydig has noted a difference between active and resting glands in his specimens. The resting glands resemble the jaw glands while the active glands show very wide lumina and connective tissue trabeculae reduced to thin sheets.

Much the same conditions appear to exist in *Crotalus*. Of the two individuals studied, one shows glands with very wide lumina filled with coagulated secretion. The connective tissue layers are very thin, widening in many places sufficiently to admit of the passage of a blood vessel. The epithelium is simple low columnar with coarsely granular cytoplasm. Nuclei are round or slightly oval, have scant chromatin and lie near the base of the cell.

The second individual shows connective tissue layers greatly thickened with the cells forming a loose network with many open spaces. The lumina of the tubules are greatly contracted and corrugated. The epithelial cells here are compressed and elongated and several layers are present as a result of the contracted lumina. These cells have dark, coarsely granular cytoplasm and a single nucleus. The nuclei are large, oval bodies with pale karyoplasm, scant chromatin and one or more distinct nucleoli. They lie at different levels though the greater number appears between the middle portion and the base of the cell. The lumina of the tubules and excretory duct contain masses of loose cells. Neutral and other granule stains fail to show the presence of secretion granules in either the active or the resting glands.

The epithelium of the duct is identical with that of the gland tubules.

Interstitial granular cells

This resting gland is characterized by the presence of two types of interstitial cells, which appear only infrequently in the active gland. The first are large conspicuous cells with coarse granules, which are irregular in size and shape. The granules are usually present in such quantities as to completely obscure the nucleus. The granules are deeply stained in all acidophilic dyes utilized, such as picric acid, acid fuchsin, congo red, erythrosin, eosin, methyl orange, orange G., aniline blue, acid green and acid violet. It was found that these granules stain more quickly when the staining solution is placed in the thermostat at 60°C. The acid radicle in such stains as Wright's blood stain and Bensley's neutral gentian affects the granules. In the former, they are stained brilliantly red, while in the latter they are stained orange. They are also stained deeply in iron alum haematoxylin.

The nuclei of these cells when seen are round or slightly oval and lie near the cell membrane. A chromatin network is seen.

These cells are irregularly distributed throughout the connective tissue septa. They are found most numerous surrounding the blood vessels. They are seen also between the epithelial cells of the tubules and in some instances, processes project out into the lumen. The majority, however, are seen in close proximity to the basement membrane at the basal ends of the cells. They are conspicuous between the epithelial cells of the excretory duct, and a few appear in the connective tissue of the mucous gland. These cells have also been observed in abundance in the connective tissue, especially surrounding capillaries, and between the epithelial cells of the vagina dentis.

This picture strongly suggests that these more or less acidophilic cells are passing from the blood vessels through the connective tissue septa and between the epithelial cells into the lumen of the gland, and consequently contributing to the elements of the secretion.

The second type of interstitial granular cells, which appear in much smaller numbers than the first type, are mast-cells and show the structure and staining reactions characteristic of mast cells.

The mucous gland

The mucous gland is entirely distinct and separate from the venom gland. It is, like the venom gland, made up of short, simple columnar epithelium. The excretory duct of the venom gland passes through the center of this gland, and the gland tubules, which lie next to it, empty into it. The main excretory duct of the mucous gland itself is, however, a crescent shaped duct and lies on the under side of the gland mass next to the capsule. Near the external opening which connects with the fang these two ducts join and empty through a common onifice.

The resting cells are tall columnar, with granular cytoplasm. The nuclei are oval with well defined nucleoli and scant chromatin. In some active cells only a small globule of mucin appears near the base of the cell. Again, the whole cell is loaded with secretion.

These secretion laden cells are scattered in the upper end of the tubule but increase rapidly in numbers as the outlet of the tubule is reached. In some tubules, near the outlet, every cell is filled with mucin.

61. *Histogenesis of the otic capsule.* (Lantern.) G. L. STREETER, Carnegie Laboratory of Embryology, Baltimore.

The cartilaginous capsule of the ear is a favorable place for studying the histological features of the growth of cartilage and its associated tissues, particularly because of two reasons; in the first place, there are, on account of the intricacy of form of the labyrinth, many kinds of cartilaginous changes found there that are necessary to accommodate its growth, including the deposit of new tissue and the removal of old tissue; and in the second place the topography is so well marked by

known landmarks that all of these changes as well as the location and direction of growth can be easily followed. From such a study one is forced to conclude that the tissues of the otic capsule are capable both of differentiation and dedifferentiation throughout a considerable period of their development. This progressive and retrogressive adaptability of the cartilaginous tissue makes possible those changes that are necessary in the growth and alteration in form of the labyrinth.

In the earlier stages the precartilag tissue abuts directly against the epithelial labyrinth. Subsequently the periotic reticulum, beginning along the central borders of the canals, becomes established and spreads at the expense of the temporary precartilag thereby forming a crescentic shaped area of reticulum entirely inclosing the membranous canal. The invasion of the reticulum into the surrounding area of precartilag is brought about, at least in the later stages, by a dedifferentiation of the latter into the former.

At the same time that the precartilag is reverting into reticulum the inner margin of the cartilage that surrounds the canal is dedifferentiated into precartilag, so that a new and more peripheral area of precartilag becomes established as the old area disappears. In this way the margin of the true cartilage gradually recedes from the epithelial canal.

In human embryos 30 mm. long the cartilaginous labyrinth has attained approximately the adult form. Its subsequent development consists primarily of an increase in size to accommodate the growing membranous labyrinth. However, if one compares the superior canal of an 80 mm. fetus with that of a 30 mm. fetus it will be found that the canal has doubled its diameter and has trebled its length and furthermore its linear curvature corresponds to an arc with a longer radius. In reality therefore the developing cartilaginous labyrinth is continuously undergoing considerable changes both in size and form. The enlargement of the cartilaginous canals and their alterations in form and position involves both the excavation of cartilage and also the laying down of new cartilage, the excavation being accomplished by its dedifferentiation into precartilag and reticulum, and the new cartilage being built up, through a precartilag stage, from the periotic reticular tissue. Throughout the entire period of growth of the cartilaginous canals the elements of this continual transformation exist along their margin. The margin during this period is in a state of temporary equilibrium and is capable of advancing or receding as the conditions determine.

The first and relatively the major part of the hollowing-out of the cartilaginous canals is complete before the perichondrium makes its appearance. The perichondrium is formed as a membrane-like condensation of the periotic reticulum which can be first recognized in fetuses of about 70 mm. CR length. In its histogenesis it is analogous to the membrana propria of the epithelial canals.

62. *The genesis of the membrana tectoria and its anatomical substratum.*

O. VAN DER STRICHT (By invitation), University of Ghent, Fellow in Cytology of the Western Reserve University.

In the present paper I should like to present some preliminary results of my investigations upon the histogenesis of the superficial epithelium of the crista spiralis, the sulcus spiralis and of the membrana tectoria of the cochlea.

I had at my disposal cochleae from pig embryos of 60.0, 93.5, 127.0, 137.0, 150.0 and 190.0 mm., from a new born dog and from the following adult mammals: bat, dog, rat and mouse, fixed and stained in various ways.

Crista spiralis. The youngest pig embryos exhibit at the surface of the future crista spiralis and in the greater epithelial ridge, an epithelial layer rather thick, described by many authors; it consists of multiple rows of nuclei, the cells of which traverse its whole breadth, but the nuclei are arranged at various heights. In reality the cells represent a simple columnar epithelium. The apices of the cells, well visible on tangential sections, figure a very elegant mosaic or pavement, the numerous fields of which are separated by the terminal bars or closing lines (Schlussleisten) and contain each of them, two central corpuscles or diplosomes.

Many authors have shown how the subjacent mesenchyme gradually penetrates between the different epithelial cells of the crista spiralis in more advanced embryos and form a special layer partially epithelial and partially connective. N. Van der Stricht demonstrated that despite this extension the primitive superficial mosaic remains intact, even in the adult organ. Never do the connective tissue elements traverse the system of terminal bars.

In order to get a true picture of the connections between the epithelium and the proliferating subjacent tissue sections tangential to the surface are needed and must be compared with transverse sections. Series of preparations show the gradual thickening of the intermediate ground substance, but in such a manner that rarely is an isolated epithelium cell completely surrounded by the connective tissue. On the contrary, the epithelial cells are arranged and pressed together so that they constitute a system of bands (or sheets) ramified and anastomosed at the surface of the smallest most axial segment of the vestibular lip, near the attachment of Reissner's membrane. The bands are more or less parallel at the surface of a more lateral and larger segment which represents the substratum of the future Huschke's teeth, the axis of the bands being directed from the axial region towards the free edge of the lip. This axis exactly represents the future furrow, separating two developed "teeth." The spaces between the parallel cellular sheets, occupied by the proliferating connective tissue rapidly enlarge, reach their definitive extension and constitute the 'teeth.'

A slightly oblique tangential section at the surface of the crista displays a series of interesting figures, varying according to the depth cut by the knife. From the surface to the depth one sees:

(a) The superficial epithelial mosaic.

(b) Cytoplasmic sheets, parallel on the lateral and anastomosed on the axial segment of the vestibular lip.

(c) A little deeper the protoplasmic bands become nucleated and their nuclei are pressed together in such a way that often no trace of cytoplasm is perceptible between them.

(b) The vascular tissue, areolar in the young, embryonic organ and fibrous in the adult.

The existence of such cellular bands suggests two special questions.

1. At first the possibility of the genesis by fusion of epithelial cells, primitively separated, of a vast syncytium, unexampled in other organs: formed by cells entirely fused along their length but absolutely distinct at their apices where I find, even in the adult persisting distinct centrosomes. Comparing the transverse with the tangential sections already mentioned I have no doubt about the presence of such a syncytial formation at least at some places of the organ. Nevertheless great caution is to be observed before drawing final conclusions for besides very demonstrative figures one sometimes observes sites where primitive intercellular boundaries are persisting partially or even entirely.

2. A second question seems to me at the present time more difficult to solve. Do these multiple nuclei so numerous in this syncytium represent those of the originally separated cells or do they partially result from the mitosis or amitosis of these nuclei? From the stages of 93.5 mm. (and probably earlier) no mitosis can be found. Should new nuclei appear later it must be admitted that they are formed by a process of direct nuclear division. Although my preparations exhibit some few signs of nuclear amitosis, I am not able to give a final answer to this question.

The structure of the greater and the lesser epithelial ridges is known. I will only point out that the greater thickening from rather early stages shows three more or less distinct segments:

1. A lateral sensorial; very small, beside the lesser ridge; in which appear the inner auditory and inner supporting cells;

2. An axial; the columnar cells of which possess a clear protoplasm and represent the future elements of the sulcus spiralis;

3. An intermediary; between the two preceding; the largest, consisting of prismatic cells, the protoplasm of which is rather dense and dark. The last mentioned cells will provide the epithelial elements covering the region of the foramina nervina.

Tangential sections at the surface of the greater ridge exhibit a very regular mosaic formed by the apices of the columnar cells with their contained diplosomes and the terminal bars separating the cellular fields.

I will not speak of the structure of the lesser ridge studied by N. Van der Stricht who gives an accurate description of its constituents and superficial mosaic.

Membrana tectoria. I have described the generating substratum of this organ, the crista spiralis and the great epithelial ridge and chiefly their superficial mosaic. The last mentioned varies somewhat in aspect according to the site. On the surface of the vestibular lip its fields are large and the closing lines are thin but a little thicker than in the areas covered by indifferent epithelium. (e.g., area of Henle's cells). In the sensorial sections (organ of Corti), they exactly represent the apices of the auditory and sustentacular cells and the terminal bars become very thick (N. Van der Stricht) although they are thin at the beginning of the genesis of the membrana tectoria.

On the surface of the greater ridge the fields are of equal size in the earliest stages but much smaller than on the vestibular lip and the terminal bars are always relatively thick. When the axial segment which first becomes detached from the membrane and inactive (Hardesty) may be distinguished from the intermediate, the fields on its surface are much smaller than in the latter segment.

This system of lines and polygonal fields interferes with the genesis of the membrana tectoria. In previous papers my pupils and I have referred to the paramount importance of the closing lines in the formation of the membrana reticularis of the auditory epithelium, of the membrana limitans olfactoria and of the membrana limitans externa of the retina. I will add here that the zona pellucida of the ovum of dog, bat and other mammals according to my preparations has a similar origin.

My preparations of the duct of the cochlea show that the terminal bars, that is the superficial intercellular cement, condensed, becoming denser and more intensively staining, take part in the genesis of the membrana tectoria in such a manner that they generate the dense part whereas the apices of the cells the more fluid part of the membrana tectoria. But the chemical composition of the dense part is quite different from that of the bars for they take up different stains.

At the surface of its matrix the membrane is formed by a system of delicate lamellae the sections of which represent a reticulum of lines, filaments, in direct continuity with the bars lying over and reproducing exactly in a tangential section the system of the closing lines. The spaces between these lamellae are filled by a paler substance which seems to be elaborated by the superficial protoplasm of each cell always provided with the centrosome. This fluid like substance and the lamellae may suffer more or less shrinkage and distortion provoked by the fixing agents so that indeed the meshes become smaller or larger and irregular.

In very successful preparations I perceive a similar but irregular network in the sections at the surface of the sensorial epithelium of the organ of Corti. I am thus induced to conclude that the membrane is formed by the same histogenetic process.

This network, first origin of the membrana tectoria, has been described by other authors but erroneously considered as produced by the cytoplasmic summits of the cells (Hardesty, Coyne and Cannieu,

Prentiss). A series of preparations fixed by the new Cajal method shows the terminal bars stained black and the lamellae of the membrane colored in the same manner.

For the wealth of bat material the study of which in the present connection has been very useful to me I am indebted to the generous interest of Mr. W. G. Marshall.

*63. *Some anatomical features of the bird hand.* (Lantern.) R. M. STRONG, Anatomical Laboratories, Vanderbilt University Medical School.

This work is part of a comparative study of bird anatomy giving especial attention to the Tubinares. The anatomy of the bird hand has been found to need more elaborate treatment than appears in the literature. Fürbringer's exhaustive work dealt especially with the brachial plexus and the muscles of the breast, shoulder, and upper arm.

On this occasion I shall present some details in the anatomy of the albatross hand, with the aid of lantern slides. The mechanism for controlling the flight feathers will be described.

What I gave called the palmar aponeurosis is a part of this apparatus and has not been described in the literature, for birds, to my knowledge. It is continuous at the radial border of the wrist with a dorsal aponeurosis. The palmar aponeurosis merges with fascias of the upper arm, and it gives off slips to the quills of the distal secondaries and most of the primary feathers. It is also attached at points to the phalanges and to the carpo-metacarpus. Further details are not given here as illustrations are needed. This work is planned for publication elsewhere later.

64. *On the nature of basal striations in salivary ducts.* JOHN SUNDWALL, Department of Anatomy, University of Kansas.

The presence of basal striations in the salivary ducts, which are characteristic of these ducts, was early noted by Pflüger, R. Heidenhain and Müller, and have been described also by Merkel, Zerner, R. Krause and others, in various animals. That these ducts are concerned in salivary secretion was maintained by Zerner, Eckhard, Milawsky and Smirnow, Solger, and R. Krause among others.

The development of new technique, particularly that for the specific demonstration of mitochondria, has made it imperative that the various glands be made the object of reinvestigation. Much investigation is now going on regarding the presence of and the rôle that mitochondria play in cell cytoplasm. Meves, Bensley, Cowdry, Duesberg, Champy, Hoven, and Arnold, among others, have contributed much to our knowledge of these structures. Whether mitochondria are directly concerned in the production of secretion substances still remains within the field of speculation, notwithstanding that Hoven, Champy, and Arnold hold that such is their special function in gland cells.

Bensley has observed that basal striations in the acinous cells of the pancreas are primarily due to mitochondrial filaments. I also have

observed that when striations were seen in the lachrymal gland, they were dependent upon the preservation of these structures. These observations naturally suggested that the basal striation in the salivary ducts might belong to the same category.

With a view of determining the nature of basal striations in the salivary ducts, I applied Bensley's acetic osmic bichromate, anilin fuchsin, methyl green technique in the preparation of salivary glands taken from the opossum. The mitochondria appear as rods or filaments in the base of the cell, occupying approximately the basal one-third of the cell and terminating as a rule at the base of the nucleus. They are arranged perpendicularly to the long axis of the cell and appear as basal rays in cross sections of the duct. Collectively the basal striation formed by the mitochondria is of the same length in the various cells that make up the salivary ducts. So that in cross sections of these ducts an even deep red fuchsinophilic border is prominent. The border is regular in width and occupies the outer third of the radii of the ducts.

The individual mitochondria making up the basal striations may be generally classified into three groups:

1. Long filaments or rods which extend the entire perpendicular length of the basal striations. As a rule these tend to taper off slightly at both ends and are thickest in the middle. However, many are approximately the same width throughout and terminate bluntly.

2. Short bacillus-like rods of irregular lengths arranged in rows.

3. Minute granules or coccus-like mitochondria arranged in rows or seen more frequently associated with the short rod forms.

Groups 1 and 2 are the types most frequently seen making up the striations. The mitochondrial rods are indeed numerous and are very closely appressed so that under low power observation the characteristic more or less red homogeneous border is noted, especially when sections of 10 or more μ are examined. Observation of sections under 5 μ by means of high power lenses—oil emersion, reveals the rows of mitochondria and individual mitochondria making up these perpendicular striations. The intermitochondrial basal cytoplasm is seen as very narrow strips between the mitochondrial rods. This undifferentiated cytoplasm appears either unstained or as slightly greenish stained clefts between the mitochondrial rods.

In the middle and proximal zones of the cell, the mitochondria are present in the form of minute granules or cocci irregularly distributed throughout the cytoplasm. In some cells they are found much more numerous than in others. Two cells may be seen side by side in one of which they are abundantly present, while in the other only a few are seen.

When the animal has been subject to pilocarpinization, as manifested by the extraordinary secretion of saliva, an interesting phenomenon is observed in the salivary ducts. The mitochondrial rods making up the basal striations show a tendency to lose their perpendicular arrangement and break up into the granular or coccus-like forms. In some

ducts all evidences of the perpendicular arrangement of the mitochondria have disappeared and the mitochondrial rods have disintegrated into granules. Granules of mitochondria, irregularly distributed, now occupy the basal portion of the cell as well as the proximal portion. The granules are more congregated than in the nonstimulated gland, so appear more numerous, and a granule-free zone of cytoplasm is noted between the mitochondria and the cell membrane.

This description of the stimulated glands represents the extreme picture of the effects of pilocarpin. In many of the stimulated glands the change in the arrangement of the mitochondria is not so marked, and in some the basal striations are only partially disintegrated.

Perhaps the chief value of these observations is the determination of the nature of basal striation of salivary ducts. That this is formed by two elements is apparent—mitochondrial rods perpendicularly arranged (the so-called filaments of Michaelis and Altmann) and an intermitochondrial undifferentiated cytoplasm (the basal filaments of Solger or the ergastoplasmic filaments of Prenant, Garnier, and Bouin). The latter are especially prominent when the mitochondrial elements have been destroyed by the fixation fluids. Bensley differentiated these two elements in his studies of the pancreas.

These observations also explain the inconstant results obtained by investigators and teachers of histology in respect to the basal striations of salivary ducts and other gland ducts. Naturally when the mitochondria are destroyed by the action of certain fixing fluids, such as those containing considerable quantities of acetic acid, alcohol, corrosive sublimate, etc., the basal striations are not distinctly conserved and consequently the sections are of little value for specific teaching purposes.

65. *Early development of the cartilaginous ethmoidal skeleton in cat.* R. J. TERRY, Washington University Medical School, St. Louis.

The first evidence of chondrification in the ethmoidal region was observed in embryos of 12 mm. (crown-rump), prepared by van Wijhe's method; in the ventral part of the future septum nasi are two deeply stained parallel streaks separated across the mid-plane by a lightly stained interval, extending forward from the travecular plate. In embryos of 5 mm. the septum is a single, high median cartilage dividing dorsally into a pair of processes, the parieto-tectal cartilages, which arch over the anterior part of the nasal cavity of each side. A second region of chondrification was found next to that diverticulum of the cavum nasi which later gives rise to the frontal air sinuses. Here a pair of paranasal cartilages is formed independently of the parieto-tectal cartilages behind which they lie. A third contribution to the early ethmoidal skeleton appears in embryos of 17 mm. as a pair of small cartilaginous plates, laminae antorbitales, at the very back of the cavum nasi on either side of the nasal septum. At this stage there is no cartilaginous ethmoidal floor. A floor is first indicated in embryos of about 20 mm. by that portion of the paraseptal cartilage which stands

in relation to Jacobson's organ and by the anterior transverse lamina. The posterior portion of the paraseptal and the nasopalatine cartilages appear relatively late. All of these cartilaginous centers, with the exception of the parieto-tectal and possibly the anterior transverse lamina and nasopalatine cartilages arise independently of one another. Nearly complete fusion takes place secondarily between the adjacent edges of the parieto-tectal and paranasal cartilages resulting in the formation of the crista semicircularis within the nose and leaving a small space, the foramen epiphaniale for the passage of the nasociliary nerve. Fusion of the neighboring edges of the paranasal and antorbital plate also occurs; at their junction internally, a small ridge is found where ethmoturbin al I is later developed. Union of the antorbital plate with the septum nasi completes the posterior transverse lamina and posterior cupola of the nasal capsule. The anterior transverse lamina is, at an early stage, continuous with the parieto-tectal cartilage, if indeed it is not originally an extension of it.

*66. *The development of the hemal channels in the central nervous system of the albino rat.* FREDERICK TILNEY and LOUIS CASAMAJOR, Departments of Anatomy and Neurology, Columbia University.

The manner in which the central nervous system acquires its vascular channels is a process presenting several distinctive features. In the first place the neuraxis is devoid of mesenchymal elements for a relatively long period in its ontogenesis. There is a boundary line between mesenchyme and neural ectoderm during this period which is sharp and unmistakable, namely the *external limiting membrane*. In consequence of this separation of mesenchyme from neural ectoderm the neuraxis is lacking for a considerable time in the angiogenetic elements necessary to vascular formation. How, therefore, are these angiogenetic derivatives of the mesenchyme acquired by the central nervous system and what, after their acquisition, are the details of the vasofactive process within the neural tube?

The material selected in the investigation of this problem was the albino rat from somite stages to term and later. As the injection method has been shown to be inadequate for the demonstration of conditions in the early development of the vascular system, this method was not employed. The study was based upon serial sections cut at 5μ after fixation in Bouin's fluid and stained according to the Mann-Regan method. Reconstructions were also made at magnifications varying from 300 to 1100 diameters.

In the hemal vascularization of the central nervous system of the albino rat, it is possible to discern two more or less distinct phases, the one characterized by the exuberant formation of blood vessels, the other, making its appearance somewhat later, characterized by the selection of the definitive vascular lines and the gradual disappearance of the excess in the earlier, exuberant formation of hemal channels. The dominant feature in the first of these phases is an *angiogenetic process* while the second phase, although still angiogenetic to a marked de-

gree, is rendered conspicuous by an *angiolytic process*. During the phase of vascularization characterized by angiogenesis several stages may be distinguished. For example, in the early somite period of development, not only the neuraxis but its immediate mesenchymal environment are lacking in any evidence of vessel formation. From this absence of all angiogenetic activity, the conditions may be described as the *prevascular stage*. This stage is limited in its duration for embryos as young as 2 to 3 mm. manifest a decided angiogenetic activity in the mesenchyme immediately surrounding the neural tube. The mesenchymal cells are collected in scattered groups which border upon the external limiting membrane. Near the center of many of these groups erythroblasts in the several stages of their evolution are seen. In many instances the hemopoietic mesenchymal elements are still in part attached to the surrounding mass of mesenchyme. In other instances the erythroblasts lie wholly detached within well-formed endothelial spaces. During this stage it is possible to recognize in the perineural mesenchyme blood islands and isolated endothelial spaces containing blood cells. Thus the vasofactive process, giving rise to isolated endothelial spaces, goes hand in hand with the formation of perineural blood islands from which latter the endothelial spaces and erythroblasts are derived. It is essential to note that at this particular period there is no evidence of vascularization in the neural tube and for this reason the conditions may properly be described as the *stage of perineural angiogenesis*.

In embryos of 3 to 4 mm. length a notable and critical change occurs. The mesenchyme assumes a definite attitude with reference to the nervous system in that many mesenchymal cells begin to make their way through the external limiting membrane into the neural tube. This wandering into the neuraxis of mesenchyme cells constitutes a veritable mesenchymal invasion. Cells of the mesenchyme, singly and in strings of two or three, make their way from the perineural mesenchyme into the neural ectoderm. These invading cells are not sprouts from the already formed endothelial spaces of the perineural mesenchyme. In many instances they proceed inward from regions in which neither blood islands nor isolated endothelial spaces are to be found. They may be seen either partially within the nervous system or their general course after entrance may be traced as strands extending toward the central canal. Coincident with this invasion there is a distinct advance in the perineural angiogenesis, for during this period the blood islands have given place to many isolated endothelial spaces, some of which have already become confluent with others in their immediate vicinity. Inasmuch as the outstanding feature at this time of development is the invasion of the neural tube by mesenchymal cells, this condition may be described as the *stage of mesenchymal invasion*.

Invasion of the tube by cells from the mesenchyme does not cease at the stage just mentioned, but continues until a much later period. Its fundamental importance in this early period lies in the fact that the neuraxis thus acquires the angiogenetic elements which serve as the

anlage for its vascular system. In embryos from 4 to 5.5 mm. in length the mesenchymal cells in the neural tube give evidence that the basofactive process is well under way. Numerous areas are observed which have all the appearances of the perineural blood islands, namely clusters of cells, some of which seem to be assuming endothelial characters while others, more central in position, present many features which ally them with the erythroblasts seen elsewhere. These latter cells are in many instances still attached to the mesenchymal clusters of which they form a part. In other areas the vasofactive process has advanced a step further and in consequence not a few isolated endothelial spaces containing free erythroblasts have made their appearance. The position occupied by the blood islands and isolated endothelial spaces within the neuraxis is noteworthy. At this time the neural ectoderm, in the myelencephalic region is eight to ten cells deep. The spaces and blood islands are situated near the central canal, being separated from it usually by two and never by more than three layers of cells. This position removes the spaces and blood islands within the neural tube as far as possible from the perineural endothelial spaces. The latter, by a process of confluence, have formed a well defined perineural plexus which, however, has no connection as yet with any of the endothelial spaces within the tube. The most conspicuous feature in embryos of this size is the inception of vascular formation within the neuraxis and the conditions may be described as the *stage of entoneural angiogenesis*.

In embryos from 6 to 7 mm. in length the number of entoneural endothelial spaces has increased and many of them have become confluent to form a relatively rich ento-neural plexus. As this plexus first takes form it presents no connection with the perineural plexus. Gradually, however, these two plexuses establish communication with each by the process of confluence. At first these communications are few in number but as the entoneural plexus increases in complexity the communications become more diffuse and more numerous until the period of full efflorescence in the vasofactive process is reached at 8.5 mm. when almost every section (cut at 5μ) shows two or more connections between the perineural and entoneural plexuses. In embryos of 8.5 mm. the vasofactive process is at its height since the entoneural plexus is then more extensive, the size of its hemal channels larger and its connections with the perineural plexus more numerous than at any other period. From this time on the entoneural plexus gradually becomes reduced in richness as well as in the size of its channels.

In embryos from 9 to 24 mm. vascular formation passes into it by actual angiolytic. This latter process confines itself to the apparent excess of the early exuberant entoneural plexus. The prominent features of the angiolytic phase may be summarized as follows: (a) The partial solution of the rich entoneural plexus during which only the more radially disposed channels withstand the retrograde process and are thus selected to participate in the permanent vascular pattern, (b) the deflorescent channels revert to mesenchyme, (c) the deposition of such mesenchymal cells among the neural elements of the central nerv-

ous system and (d) the breaking down of many erythroblasts excluded from the circulation. From our observations we have drawn the following conclusions:

1. The development of the hemal channels of the central nervous system depends upon the formation of two separate plexuses, i.e., the perineural plexus and the entoneural plexus.

2. The perineural plexus is developed from the perineural mesenchyme first as a series of blood islands which give rise to isolated endothelial spaces, the latter in turn becoming confluent to form a plexus.

3. The entoneural plexus is developed from derivatives of the perineural mesenchyme, which, in the early stages, invade the neural tube, there giving rise to blood islands. From these blood islands are formed isolated endothelial spaces which become confluent to form a plexus.

4. The perineural and entoneural plexuses establish communication with each other by a process of confluence.

5. The entoneural plexus attains its highest development in the relatively early stages and then undergoes deflorescence. As a result of this latter process a considerable portion of the primitive vascular tissue within the neural tube reverts to mesenchyme cells which remain as constituents of the neuraxis.

67. *On the pineal region in human embryos.* JOHN WARREN, Harvard Medical School.

The object of this communication is to call attention to three special features in the development of the pineal region in human embryos.

1. *The primary arches in the roof of the forebrain.* In an embryo of 10 mm. all these arches are clearly differentiated. A low velum separates a well marked paraphysal arch from a relatively short and thick-walled post velar arch. The epiphysal arch is sharply defined with also rather thick walls and is succeeded by a relatively long pars intercalaris. This part of the brain roof forms a low arch in the posterior end of which the posterior commissure can be seen. The commissure seems to appear in this portion of the forebrain before invading the roof of the midbrain. In an embryo of 15 mm. the arches are more fully developed. A slight median thickening in the paraphysal arch marks the anlage of the paraphysis. The posterior commissure now occupies a larger part of the pars intercalaris and has developed backward into the midbrain. The primary arches as first described by Professor Minot in *Acanthias* can therefore be demonstrated in human embryos and in addition the pars intercalaris forms an arch of relatively great length as compared with its appearance in lower vertebrates.

2. *Paraphysis.* In the embryos of the Harvard Collection the earliest trace of the paraphysis can be noted as a slight thickening in the paraphysal arch in the embryo of 15 mm. mentioned above and in two others of 16 mm. No traces could be found however in any of the embryos of from 17 to 22 mm. with the possible exception of one of 19 mm. In an embryo of 23 mm. sagittal series it appears as a tiny

hollow elevation similar to its earliest form in lower vertebrates. In another embryo of 23 mm. transverse series it is a little larger and in one of 25 mm. it forms a good sized oval outgrowth with a cavity opening into that of the brain in front of the velum. Above this stage it could be demonstrated in an embryo of 31 mm. as a mere tiny elevation and also in one of 36 mm. where there was a slight cavity attached by a solid stalk to the brain wall. The oldest embryo in which any trace could be made out was one of 44.3 mm. where a tiny conical elevation in the paraphysal arch was all that could be seen. The paraphysis therefore does exist in certain human embryos but it is a rudimentary and inconstant structure. A short but well defined paraphysal arch could be followed in all the embryos studied.

3. *Post velar arch.* A complicated prolongation of the anterior end of this arch just behind the velum forms a striking feature in many embryos. This outgrowth appears either as a median projection or as a bilateral formation on either side of the median line coming into intimate relation with the vessels over the brain roof. As the outgrowth becomes more complicated, tubules are given off in a rather bewildering manner, which may become detached and appear as blind vesicles buried in the midst of this tubular formation. The paraphysis is more or less covered by this projection which overhangs to a large extent the paraphysal arch. The formation begins in embryos of 23 mm. as a simple median outgrowth. In an embryo of 25 mm. the outgrowth is a double one. In an embryo of 31 mm. two closed vesicles can be seen on either side of the middle arch, but still in contact with the brain wall. In an embryo of 36 and 44 mm. where the formation is extremely complex, one or more of these vesicles are found completely detached from the brain. Their walls are usually thinner than those of the other tubules among which they lie. Attention is called to this formation especially to the vesicular portion as it is such a striking feature in all the embryos from 23 mm. up to 44 mm.

68. *Progressive movements in decerebrate kittens.* LEWIS H. WEED, Anatomical Laboratory, Johns Hopkins University.

In a series of forty kittens subjected to decerebration, different reactions of a rhythmic character were obtained. In all, an essentially similar ablation was performed with removal of cerebral hemispheres and basal ganglia. The brain-stem was cut through just anterior to the superior corpora quadrigemina, sloping forward in the line of the bony tentorium. The kittens continued to breathe spontaneously and as soon as the anesthesia had passed away, showed active reflexes and rhythmic progressive movements.

Differing from the reactions of adult animals, these decerebrate kittens did not exhibit an invariable extensor rigidity. All, however, gave typical scratch reflexes; they responded to both dorsal and ventral excitation, and reacted to trauma to the tail. In general the animals after decerebration were much more active than are the customary adult preparations. Practically all of these animals showed

rhythmic progressive movements; they seem to fall into two classes in this reaction, the groups being determined by the length of time the beats continued.

Twelve of these decerebrate kittens, on recovery from the anesthesia, showed, when suspended, typical prolonged walking movements of all four legs. These rhythmic progressive beats could be initiated by almost any stimulation and were continued for varying periods. In some of the animals the movements, after the initial excitation, were prolonged uninterruptedly until exhaustion occurred; in others the movements ceased after a few minutes. In the more active kittens, a smooth surface placed beneath the four feet of the animal occasioned continued progressive movements.

Separated from this group of kittens which showed prolonged rhythmic movements of progression are the other kittens of the series. These on appropriate excitation, made rhythmic movements lasting not over thirty seconds after the cessation of the stimulus. While the differentiation of the two groups is wholly arbitrary, the reactions of the animals are so different in this temporal relationship that the two classes seem indicated.

The reactions of the group of decerebrate kittens showing prolonged progressive beats are those of an adult cat in which only the cerebral hemispheres have been removed. The animals of the second group, however, are somewhat more active than are adult decerebrate cats. In the kittens of the first group, an extensor rigidity was absent in all but three; in these, a questionable rigidity occurred soon after the ablation and was quickly abolished by the onset of the rhythmic beats of all four legs. As the tendency to progression decreased, extensor rigidities became more pronounced, affecting in some only the forelegs. A reciprocal relationship between the prolonged progressive movements and the extensor rigidities has been indicated by these experiments.

The kittens which when decerebrated showed the phenomenon of prolonged progression varied in age from one hour to sixteen days (in length, from 150 to 220 mm.). Kittens of approximately the same age from the same litters in general gave similar reactions but considerable variation could be noticed. In any one litter, as the kittens grew older, the tendency toward prolonged progression grew less and the likelihood of extensor rigidities grew greater. Age alone, however, is, as judged by these observations, not an index of the reactions.

69. *The coronary vessels of the heart of the 20-mm. pig embryo.* (Lantern.) THOMAS FOSTER WHEELDON, Harvard Medical School. (Introduced by Franklin P. Johnson.)

The present article is an extract from a larger paper entitled "The heart of the 20 mm. pig embryo." It is a study which was carried to completion at the University of Missouri under the direction of Professor Johnson.

Arteriae coronariae. As in the adult, the coronary arteries are two in number, a right and a left. They appear as small rounded vessels

with relatively thick walls. They spring from the superior aortic sinus and the left inferior aortic sinus respectively, and are distributed almost entirely to the heart, but a few small branches are given off to the roots of the great vessels.

The left coronary artery gives off a branch of particular interest which accompanies the atrio-ventricular bundle through the moderator band, and breaks up into capillaries in the right wall of the right ventricle.

Attention must be called, also, to a plexus to be found on the diaphragmatic surface of the heart. This plexus is formed by the anastomosis of the terminal branches of the right and left coronary arteries.

Sinus coronarius et venae cordis. Owing to the fact that the venal hemi-azygos of the pig opens into the coronary sinus, the relations of the entering cardiac veins are somewhat different from those found in man. There is, strictly speaking, no definite coronary sinus, for the cardiac veins all enter the terminal portion of the vena hemi-azygos. This portion of the vena hemi-azygos, which lies in the inferior portion of the coronary sulcus, is somewhat enlarged, and represents the coronary sinus of the human heart. In my description, I have used the term 'coronary sinus' to designate the terminal portion of the vena hemi-azygos.

The coronary sinus receives five large veins. They are from left to right; the vena hemi-azygos, the great cardiac vein, the inferior cardiac vein of the left ventricle, the middle cardiac vein, and the small cardiac vein. In addition, it receives the smaller anterior cardiac veins. It empties into the sinus venosus just ventral to the inferior vena cava. There is as yet no subdivision of the valve of the venous sinus to mark out the Thebesian valve, which in the adult pig guards the orifice of the coronary sinus.

The small cardiac vein, instead of arising as a single trunk as it does in the adult pig, arises as two definite vessels, one of which receives branches from the right atrium, while the other collects blood from the right wall of the right ventricle.

Little seems to have been published on the development or observation of the coronary vessels in embryos younger than 20 mm., and it was quite unexpected to find so complete a system in a pig of this size.

70. *Study of a human spina bifida monster with encephalocèles and other abnormalities.* THEODORA WHEELER, Carnegie Institute of Embryology, Baltimore.

The specimen was accompanied by only a meager clinical history; the child was illegitimate, was born spontaneously at full term, and lived only a few hours.

The external form is fairly typical of a class often called inencephalic monsters. There is extreme dorsal flexion and shortening of the trunk. The head is drawn back close to the sacral region. At the back of the head three encephalocèles project. The shoulders lie very high, pushed close to the cheeks. The neck is obliterated, the chin

and chest lying in one plane. The ears are distorted; the anthelix is pushed out so that it is very prominent; the tragus is shifted medially and upwards so that it lies opposite the concha; the antitragus lies below the tragus pushed against the cheek; the lobule is narrowed. These deformities are evidently caused by pressure on the external parts of the ear and twisting of these parts during their early development by the backward bent head and the shoulders which lie close to it on each side. In the region of the encephaloceles there is some normal scalp and also various cutaneous anomalies. On the lower border of the left sac there is a rounded bleb of porous, wrinkled skin, 1 cm. in diameter. A section through this region shows the bleb to lie over a funnel shaped canal which extends into the cerebro-spinal cavity. The covering of the central encephalocele is formed largely by a leather-like tissue. This consists of a very vascular connective tissue over the surface of which is a very thin layer of epidermis. There are numerous sweat glands in this region but no hair follicles. Near the tip of the sac there are two naevi; these consist of areas where the epidermis is lacking and the vascular tissue extends to the surface.

A sagittal section was made of the specimen. The central nervous system was removed and a wax model made of the cerebro-spinal cavity. The specimen was then dissected.

The skeleton shows marked maldevelopment. The arches of all the vertebrae are defective; they are open posteriorly and are flattened outwards. In the cervical and thoracic regions the bodies of the vertebrae are fused, shortened, and dorsally flexed, so that the spine is bent almost double. The occiput rests on the gaping vertebral arches and actually fuses with them on both sides. The lower two-thirds of the squamous portion of the occiput is defective. This forms a very large foramen magnum through which much of the brain has slipped. There is slight scoliosis of the vertebral plate. The lengths of the presacral vertebral parts are: cervical 15 mm.; thoracic 45 mm.; lumbar 43 mm. Comparing these with Aeby's figures for average lengths of these parts in the normal newborn, cervical 45.1 mm.; thoracic 83.9 mm.; lumbar 47.5 mm., it is seen that while the lumbar portion of this specimen is of nearly normal length, its cervical portion is less than half, and its thoracic portion a trifle more than half that long. The vertebral bodies themselves have become somewhat widened. The ribs have undergone considerable disturbance. There are twelve ribs on each side. On the right the first six are fused near their origins; the second rib terminates at the end of its proximal third in a plate of bone joined to the first and third ribs. On the left the fifth to ninth ribs are crowded together in their proximal half; the fifth and sixth ribs have but one costal cartilage between them. The sternum is well formed. There are but six costal cartilage connections on each side. There is a persistent episternum. The two scapulae are defective along their vertebral margins. The left scapula is a much wider bone than the right owing to a prolongation of the medial extremity of the spinous process. This prolongation is joined to a rod of bone which in turn is attached

to the everted vertebral arches of that side. This stimulates a condition frequently associated with Sprengel's deformity, congenital elevation of the scapula. The cause of the hunched position of the shoulders, so prominent externally, shows clearly in the skeletal condition. The cervical and upper thoracic vertebrae lie crumpled to half their normal extent under the scapulae and completely change the normal relationships of these parts.

The following muscles have undergone disturbance. The trapezei are reduced to thin strap-like bands. The rhomboidei are shortened to 3 mm.; they arise from connective tissue over the everted arches of the thoracic vertebrae and are inserted in fascia along the inferior vertebral borders of the scapulae. The posterior superior serati could not be identified. The anterior serati are probably represented by scattered tissue on both thoracic walls. The sacro-spinalis and short back muscles lie as two separate muscle bundles, one on each side of the everted vertebral arches.

The soft palate is not formed. There is a bilateral anlage of the uvula on the sides of the pharynx. The right lung is formed of but one lobe.

The central nervous system is very much distorted. The three encephaloceles lie below the foramen magnum. There is considerable cerebral tissue in the left and middle encephaloceles while the one on the right side contains cerebellar tissue. At the base of the brain, of which a large part has slipped below the foramen magnum onto the thoracic vertebrae, the cranial nerves can all be identified; they are much elongated. Owing to a Z-shaped bend of the cord and brain stem the fourth ventricle has been completely inverted and lies on top of the spinal cord. From the floor of the fourth ventricle some cerebellar tissue is drawn back as a flattened sheet to join the rest of the cerebellar tissue in the right encephalocele. The spinal nerves though much crowded are all present, emerging from a flat spinal cord.

71. *Variations in the wall and epithelium of the stomach and esophagus in normal distention.* (Lantern.) H. O. WHITE, Anatomical Laboratory of the College of Physicians and Surgeons, University of Southern California, Los Angeles.

From observations and comparisons derived in the laboratory course of normal histology during the least few semesters, it appeared that the marked changes evident in the musculature and epithelium of the stomach and esophagus merit detailed investigation. To accomplish this the stomach and esophagus of two freshly killed cats were utilized in the normally distended and collapsed conditions. In order to fix the organs in a state of approximately normal distention, saturated aqueous solution of mercuric chlorid was injected into them, and after securely ligating to prevent the outflow of the fluid, were immersed in the same solution for further fixation. Longitudinal and transverse frozen sections from the contracted and distended organs were made, after proper washing in running water and subsequently in iodized

70 per cent alcohol. Details of histological variations were best obtained by staining the sections with hematoxylin and Picro-acid-fuchsin. Preference was given to the latter stain due to its brilliancy with which it brings out muscle and fibrous tissue. The following results were obtained after measurements taken in micra.

Under the same pressure, the esophageal wall in nearly normal contraction and distention, shows a greater decrease in thickness than that of both parts of the stomach wall under the same conditions.

Comparing the limit of distensibility of the stomach and oesophageal walls, under the same pressure, I found that it is more definite in the latter than in the former, due in all probabilities to the greater amount of supporting and muscle tissue, and also to the varied directions of distribution of the muscle layers of the stomach. In the mucous coat the papillae-like projections of the tunica propria, or corium upon which the epithelium of the esophagus rests are, in consequence of normal distention entirely obliterated, to such an extent that the tunic represents a smooth layer of areolar tissue supporting an equally smooth layer of stratified epithelium, and the tunic is markedly reduced in thickness. The tunica propria of the normally distended stomach, though very appreciably reduced in thickness, does not lose its characteristic appearance, due undoubtedly to the greater abundance of areolar tissue in that tunic and also to the greater folding of the entire mucosa of the stomach.

While in the contracted state only the innermost cells of the epithelium of the esophagus are flattened, and the flatness gradually changing as the middle layers are approached until finally the outermost layer of cells is rather of a simple columnar shape and the nuclei round or oval, in the normally distended esophagus, not only the familiar shape of the entire epithelium changes, but is also much reduced in thickness, every cell is flattened out and hence elongated; the nuclei in consequence assuming a distinct spindle-shaped appearance.

May not this diminution of the epithelium in thickness (about 65 per cent) together with the flattening of the cells in consequence of normal distention possibly suggest an actual gliding of one cell upon the other, and hence a displacement to some extent? As to the epithelium of the stomach, while the entire mucosa is reduced in thickness (about 40 per cent) in normal distention under the same pressure as in the esophagus, the epithelium itself is only slightly compressed, due probably to the fact that the tunica propria, normally prolonged into the lumen of the stomach above the gastric glands supporting the single layer of columnar epithelium, forms projections of considerable length and these, during normal distention, become imbricated, thereby preventing material changes in the epithelium of the viscus.

The musculature of normally distended stomach and esophagus share relatively an equal reduction in thickness, the longitudinally disposed muscle layers, however, participating more in the thinning process than the circular layers, due evidently to the fact that normally the former is much thinner than the latter. On the other hand,

since the muscularis mucosa of the esophagus normally does not form a complete continuous layer, hence the fasciculi of this tunic, in normal distention, become widely separated, while the same tunic of the stomach, because of its continuity as a complete layer from one end of the stomach to the other, is only appreciable reduced in thickness (about 15 per cent) under identical conditions.

*72. *Some peripheral relations in the cranial nerves of reptiles.* WILLIAM A. WILLARD, Department of Anatomy, University of Nebraska, College of Medicine.

This paper deals with the peripheral distribution of the preauditory cranial nerves of the common garter snake, *Eutania sirtalis*, with some comparisons with the same region in the lizard, *Anolis carolinensis*, which has previously been described. The points presented represent a partial report on the study of the Ophidian head which embraces all the cranial nerves with the attempt to analyze them into their functional components. While the following descriptive account is inclusive of the main points only, it is based upon a detailed study of two excellent series of sections, one of a full grown specimen the other of a young snake about six inches in length. The only noticeable difference was one in myelination and this was so slight that it may be due to technique instead of difference in age.

The nerves to the muscles of the eyeball are well developed and their superficial origin from the brain corresponds to the condition found elsewhere. Their course to the orbit is intracranial. They converge to unite with each other and with the ophthalmic branch of the trigeminal. The identity of the IV nerve is preserved in section although included in the same sheath with the others. That of III and VI is entirely lost in V for a short part of the course. At the level of the posterior side of the orbit this common trunk again resolves itself into its component parts, VI and part of III first separating from the dorsal side of the trunk to innervate the posterior and inferior rectus muscles respectively. The remainder of III still closely applied to the ventral side of V is marked by the deeper impregnation of its fibers. As this part separates from the ophthalmic the short root of the ciliary nerve is given off from the latter. A segregation of unmyelinated and very lightly myelinated fibers which make up this root is observable in the ophthalmic portion of the common trunk before III separates from it. No direct contribution of fiber from III is recognized either before or after its separation from V. The ciliary root passes at once into the small ciliary ganglion from which a single ciliary nerve passes into the eyeball. The muscles of the orbit are limited to the four recti and two oblique, there being no accessory muscles. The six muscles are innervated in the usual manner.

The trigeminal nerve is represented in the snake by two anatomically distinct nerves. The ophthalmic nerve has a course cephalad within the bony and membranous cranium to a point opposite the middle of the orbit where it gives off a ramus frontalis to the interorbital integ-

ument while the remainder of the nerve as the ramus nasalis after its separation from III and VI, and from the ciliary root has the same distribution as in *Anolis* to the epithelium of the nasal capsules and the integument of the preorbital region. It sends no fibers to the mucous membrane of the mouth. The ophthalmic ganglion is large and its root enters the brain just cephalad to the roots of the maxillo-mandibular division of the trigeminal. The maxillo-mandibular division of the Gasserian ganglion is relatively about twice the size of the same structure in the lizard and the nerves entering the ganglion correspondingly large. The maxillary ramus is formed by the union of an infraorbital and a superior labial branch. The first innervates the integument covering the superior labial glands, the second, in combination with the palatine branch of VII is limited to the mucous membrane of the palatine and maxillary regions of the mouth.

The mandibular ramus after giving off nearly all of its motor fibers to the muscles of the head, passes into the bony mandible. In its cephalad course it divides its fibers in a manner similar to that of the maxillary ramus, between the integument and the mucous membrane of the mouth. In addition a large lingual branch takes a recurrent course to reach the base of the long tongue sheath. With this lingual division an extremely fine chorda-tympani nerve unites.

The geniculate ganglion and the facial nerve when separated from the trigeminal, as is only possible by means of sections, present about the same condition as that found in *Anolis*. The two component roots are distinguishable within the brain. They leave the cranium through a special foramen, which is covered externally by the mandibular wing of the Gasserian ganglion. This results in the fusion of the geniculate with the latter ganglion, although its limits are clearly marked in the sections. From the geniculate ganglion a fine fibered palatine ramus passes cephalad intracranially, and a mixed fine and coarse fibered hyomandibular ramus passes caudad. The palatine ramus does not give off any branches until after it has been united anterior to the orbit by means of sympathetic ganglia with the median or infraorbital branch of the V, and there seems to be evidence in the large number of unmyelinated fibers which it contains that it represents, in part, a sympathetic pathway between these ganglia and the brain. The hyomandibular ramus gives off nearly all its fine fibers, most of which are not myelinated to form a communicating ramus with the lower sympathetic ganglia. This ramus is not joined as in the lizards by an external communicating ramus from the orbital plexus. The few remaining fine fibers in the hyomandibular ramus are given off some distance caudad. This extremely small ramus enters the lower jaw and eventually joins the lingual branch of the mandibular ramus of the V. It has been termed the chorda-tympani on the basis of its course and connections, although it has the appearance of a fine unmyelinated sympathetic ramus. There is no middle ear chamber here to modify its course as a post-trematic branch and its position is relatively farther caudad than in the lizard.

The groups of sympathetic ganglion cells which in other reptiles have been included under the terms, palatine, infraorbital, ethmoidal and mandibular ganglia are recognized in the snake in increased degree and in addition other ganglia are found not appearing in the lizard. The ciliary ganglion and nerve are smaller owing to the less specialized development of the eye.

The cutaneous sense organs are dermal corpuscles projecting into the epidermis. These are very abundant along the jaws. Special epithelial sense buds are found in the mouth along the palatine and maxillary and mandibular dental areas. These have the appearance of taste buds but their undoubted innervation by fibers carried in the branches of the trigeminal nerve suggests the possibility of a tactile function. Further study may disclose different types of sense organs in the mouth.

**73. A new method for the study of the development of the lymphatic system.* G. B. WISLOCKI, Department of Anatomy, Johns Hopkins University.

A clear idea of the origin and growth of the lymphatic system was first obtained by Sabin, who combined the study of sections of embryos with the injection of the lymphatics with india-ink. This conception comprises the fundamental truths, that the first lymphatics arise by budding from the veins, that the lymph-channels grow from center to periphery (the theory of lymphatic and non-lymphatic zones) and lastly, that the lymphatic capillaries are closed channels lined by endothelium which grow by sprouting and budding. The theory of the venous origin of the lymphatic system is in direct accord with the earlier work upon the development of blood-vessels by His, Hoyer, Prévost, Rouget and Mall and with the work upon the nature of lymphatics by von Recklinghausen and upon their growth by Ranvier.

Schwann's discovery of the vascular channels in the tadpole's tail made possible the first studies of living growing vessels, and since his time the tails of amphibian larvae have been studied again and again in hope of solving the questions of the mode of growth of lymphatics. The difficulty of this method of studying the growth of lymphatic vessels is shown by the diversity of the views to which such a study has led different observers, and it was not until Clark devised an anaesthetizing chamber that accurate and useful observations could be made. He was able to show definitely that lymphatic vessels grow by sprouting, and not by the addition to them of changed blood-vessels or tissue-spaces or the transformation of mesenchymal cells into endothelium, and his observations tend to support the argument for the venous origin of the lymphatics system.

In spite of the enormous amount of evidence in its favor, there are many who are unwilling to accept the theory of the venous origin of lymphatics as outlined by Sabin and her co-workers. It seems pertinent therefore at this time to report briefly and present a few of the results of a new technique for studying the growth of lymphatics,

which, combined with the Clark method and the study of sectioned material promises to throw additional light upon the growth and origin of lymphatics.

The method consists in vitally staining amphibian larvae and observing the lymphatics, whose endothelium stains quite specifically intra vitam with acid azo-dyes, by means of a Clark chamber and in sections.

Frog eggs were collected and brought into the laboratory, where they were hatched and the larvae kept in aquaria until used. Initially *Rana temporaria*, *Rana catesbiana*, *Hyla pickeringii* and a species of *Amblystoma* were employed and although similar results were obtained with all the species, the two former are now used exclusively because they have proven more satisfactory. The larvae were transferred a few at a time, at short intervals after hatching, to finger-bowls containing solutions of trypan-blue (the most easily obtainable diazo body) in ordinary tap-water, ranging in strength from 1:600 to 1:1000. The animals were kept in solutions of trypan-blue of these strengths for many weeks without noticeable impairment of their vitality. The details of the preliminary trials are omitted here for brevity.

The tadpoles were examined daily under the microscope in a Clark chamber for the appearance of the dye in the tail. The first trace of dye in the larvae were noticed about the fourth or fifth day, the amount rapidly increasing. From the time of its first appearance it was evident that the aggregates of coloring-matter occurred within the living endothelium of the lymphatic vessels, and by the end of ten days the stain had accumulated to such an extent, that the entire lymphatic system was brilliantly outlined in blue. The dye stained the lymphatic endothelium only, for there was no trace of blue in the endothelium of the blood-vessels, or in the circulating blood-elements, nor did the mesenchyme cells or wandering cells apparently phagocytise any of the color. There would seem to be a specific ability on the part of embryonic lymphatic endothelium to ingest dispersions of vital dyes, a specificity which furnishes us a means of distinguishing accurately lymphatic endothelium from mesenchyme or venous endothelium. Attempts to differentiate lymphatic endothelium from mesenchyme have been made before by Clark, in chick embryos, and by Kampmeier in the toad, but their criteria for differentiating the two tissues are not widely applicable and therefore are not of great practical value.

Sections of the stained animals show that the lymphatic system in its entirety had absorbed the dye, and that, as in the tail, vascular endothelium, mesenchyme cells and blood-elements were everywhere unstained. The only other tissues which seemed to store the dye in appreciable amounts was the liver, the Kupffer cells to a marked degree, the liver parenchyma to a lesser degree. Occasionally the trypan-blue was observed in groups of pyrrol-cells occurring in the mesentery, this however was by no means constant.

The absorption and excretion phases of vital staining in these larvae the author discusses in another paper. From the diffuse blue staining of

the intestinal tract it would seem most likely that absorption occurred in that region, as there is no evidence at present pointing to the epidermis or gills as a portal of entry. Excretion of the dye by the kidney-tubules and through the mucosa of the gill-arches was demonstrable.

In the living tadpole's tail, in a Clark chamber under the oil-immersion lens, the nuclear areas in the lymphatic-walls were seen to be free from stain, the dye-granules corresponding in distribution to the granular zone of the cytoplasm and extending some distance into the cytoplasmic filaments and pseudopods. The growing lymphatics were readily studied, as the lymphatic endothelium was distinguishable at all times, owing to its characteristic stain, from mesenchyme cells or vascular endothelium. The observations of Clark on the mode of growth and nature of lymphatic sprouts and tips have been repeated and verified by this method. It is to be hoped that the method will further lend itself to the solution of the problem of the growth of the thoracic duct in amphibian larvae.

DEMONSTRATIONS

1. *The cellular changes in the prostate gland of the albino rat after castration.* W. H. F. ADDISON and R. D. SPENCER, University of Pennsylvania.
2. *The origin and fate of the osteoclasts. Microscopic preparations and illustrations in color showing how osteoclasts arise from osteoblastic synticia, grow by incorporating osteoblasts and bone cells, and, in part, ultimately degenerate.* L. B. AREY, Northwestern, University Medical School.
3. *The 'ultimobranchial bodies' in various developmental stages of pig embryos.* J. A. BADERTSCHER, Indiana University School of Medicine.
4. *Studies of the cortex of the sheep brain.* CHARLES BAGLEY, JR., Phipps Psychiatric Clinic, Johns Hopkins University.
5. *Sections of folded or U-shaped embryos.* W. M. BALDWIN, Albany Medical College, Union University.
6. *Dissectible blotting paper models of various stages of development of the forebrain of Amia.* CHARLES BROOKOVER, University of Arkansas Medical Department, Little Rock.
7. 1, *Models illustrating the development of the mammalian heart.* 2, *Models illustrating the development of the mammalian vertebrae in the membranous and cartilaginous stages.* ALFRED J. BROWN, Anatomical Laboratory, Columbia University.
8. *Histological differences between certain muscles of the cat.* H. HAYS BULLARD, Johns Hopkins Medical School.

9. *Models of the brain of normal and operated Amblystoma larvae to show the morphogenesis of the lateral hemispheres.* H. SAXTON BURR, Yale University School of Medicine, New Haven.

The models here demonstrated were made from sections of normal *Amblystoma* larvae, using with one exception blotting paper instead of wax plates. The models show briefly that in the morphogenesis of the lateral hemisphere the following steps occur:

In the first place the lateral wall of the cephalic end of the neural tube becomes greatly thickened. An increase in area of this thickened portion brings about an infolding of the anterior wall in the region just dorsal to the paraphysis thus forming the first sign of the velum transversum. A continued growth results in the folding out laterally of the superior margin of the thickened area. At first this folding is most prominent dorso-laterally but soon the evagination progresses not only dorso-laterally but anteriorly beyond the limits of the original neural tube. In this manner is formed the primitive lateral ventricle which is connected with the cavity of the neural tube by the wide open inter-ventricular foramen. We have formed thus a lateral hemisphere whose outer wall consists of a much thickened ovoid mass connected to the neural tube by a thin lamina of neurogenic tissue.

Concomitant with the in-growth of the olfactory nerve from the nasal placode, the medial wall of the ventricle begins to increase in thickness, progressing posteriorly from the anterior pole. At the same time the thin layer of tissue connecting the dorso lateral portion of the hemisphere with the neural tube is in turn thickened until there is formed a hemisphere essentially like the adult, consisting of a ventro-lateral ovoid mass, the corpus striatum, a superior curved lamina, the primitive pallium, and a medial thickened wall.

One model shows a stage in the regeneration of the lateral hemisphere which had been removed by an operation which left the nasal placode in place. It is interesting to note here that the regenerating hemisphere passes through essentially the same course of development as has been described above for the normal.

10. *The innervation of the vertebrate digestive tube (methylene blue intravital staining).* F. W. CARPENTER, Trinity College, Hartford.
11. *Lipoid structures appearing in the corpus luteum cells of swine after fixation with aqueous solutions.* GEORGE W. CORNER, Hearst Anatomical Laboratory, University of California.
12. *Haematopoieses in the yolk sac of birds.* VERA DANCHAKOFF and CLAYTON SHARP, Columbia University.
13. *Lymphoid hemoblasts of the adult spleen.* VERA DANCHAKOFF, Columbia University.

14. *Intra vitam staining with the acid colloidal dye pyrrholblau:*

1. Sections of doubly ligatured femoral vein of rat showing polymorphonuclears and lymphocytes with the 'dye granules.'
2. Sections of tissue at the site of intramuscular injection, showing polymorphonuclears and lymphocytes containing the dye granules. HAL DOWNEY, Department of Animal Biology, University of Minnesota.

15. *Chondriosomes in fish embryos.* J. DUESBERG, Carnegie Institution, Department of Embryology, Baltimore.

Sections of embryos of *Fundulus heteroclitus* and *Morone americana* (white perch). The embryos were fixed with Bendas or Regaud's fluid, the sections stained with iron-hematoxylin or Bensley's fuchsin S-methylgreen. Chondriosomes, either in form of granules or of filaments, are present in the cells of all tissues.

16. *Further preparations showing the effect of vital stains on the cells of the corpus luteum, the theca folliculi, the granulosa, and the zona pellucida of the mammalian ovum.* HERBERT M. EVANS, University of California.

17. *A laboratory aid for the study of neuro-anatomy.* J. H. GLOBUS, Cornell Medical School, New York City.

A series of sketches, outlines and diagrams of the more important structures of the brain and spinal cord are incorporated in the form of a semi-completed atlas and used in the course of neuro anatomy at Cornell Medical School.

The sketches are made from carefully dissected specimens and bring into view the more important gross structures of the central nervous system. The task of labeling such structures, however, is left to the student who is, thus, required to make careful observations in order that he may label the drawing correctly.

The outlines are drawn by the aid of the projectoscope from selected transverse and longitudinal sections of the brain stem and spinal cord. The enlargement is uniform throughout and only the larger and rather easily identified structures are included in the outlines. The student from the section under his observations draws in the more minute and less evident structures. Here again the task of labeling is left to him, which demands a careful study of the given section in order to identify and label structures correctly.

A limited number of diagrams are added. They enable the student to plot certain long tracts as he traces them through the various levels of the spinal cord and brain stem. Thus a daily record is obtained of the course taken by tracts studied, and in the end isolated facts are brought together.

The first drawing of the series is completely filled in and labeled and serves as a model for putting in details in the remaining outlines. The

nuclei of gray matter are represented by groups of irregular triangles in red and the tracts by black dots of uniform size where fibers are cut transversely and black lines of variable thickness if they are cut longitudinally. The labeling is done by subdued lettering and lines.

At the end of the course the student will have a collection of correct drawings illustrating the more important structures of the central nervous system.

18. *Microscopic preparations of endothelium and wandering connective tissue cells as seat of origin of hemoglobin.* J. H. GLOBUS, Cornell Medical School.
19. *Microscopical preparations showing various phases of excretory function in selachians.* E. R. HOSKINS and MARGARET MORRIS, New York and Yale Universities.
20. *Casts of eleven gorilla brains.* A. HRDLÍČKA, Smithsonian Institution.
21. *Genetic factors leading to the development of mammalian pulmonary asymmetry. Sections, corrosions and reconstructions.* GEO. S. HUNTINGTON, Columbia University.
22. *Effects of inanition upon the structure of the hypophysis in the albino rat.* C. M. JACKSON, Institute of Anatomy, University of Minnesota.
23. *Specimens showing the later development of the lobules of the pig's liver.* FRANKLIN P. JOHNSON, University of Missouri.
24. *Methods of mounting sections in gelatine.* J. B. JOHNSTON, University of Minnesota.
25. *Microscopic preparations of mongoose, pig and turtle embryos, to show endothelial hemoblasts and aortic cell clusters.* H. E. JORDAN, University of Virginia.
26. *A demonstration of the sense cells of the retina, olfactory mucous membrane and taste-buds.* H. M. KINGERY, Cornell University Medical College, Ithaca.

The method of making these preparations is similar to that employed in obtaining a Weigert copper hematoxylin preparation. The tissue was fixed in copper-dichromate-sublimate-acetic mixture and afterwards mordanted for four to six days in copper dichromate solution. Weigert's copper hematoxylin stain was used on paraffin sections. The method seems to pick out the sense cells specifically, perhaps due to their reducing action of the dichromate in solution.
27. *A simple method of brain dissection.* P. E. LINEBACK, Atlanta Medical College, Emory University.

28. *Preparations of vitally stained bone and pathological calcareous deposits.* C. C. MACKLIN, Johns Hopkins Medical School, and the Wistar Institute of Anatomy and Biology.

29. *Radiograph prints of mesial sections of horse heads and also prints of limbs.* H. S. MURPHEY and J. D. GROSSMAN (introduced by C. R. Stockard), Department of Veterinary Anatomy, Iowa State College, Ames.

1. 280 day fetus; 2. 308 day fetus; 3. 340 day fetus (end of term); 4. 7 month foal; 5. 23 months; 6. 4 years 10 months; 7. 16 years old; 8. Old horse injected naso-lacrimal duct; parotid duct and infraorbital canal wired. 9. Vessels injected; 10. 'Poll' from above; 11. Miscellaneous views from above heads with shot in the sinuses.

1. Lateral cartilage of the third phalanx, covered by tinfoil to show extent; 2. Mesial section of metacarpus and digit; to show extent of bursae, vaginal sheathes and joint capsules marked by lead glass; 3. Lateral view of injected vessels of digits from thoracic and pelvic limbs; 4. Anterior and posterior views of No. 3; 5. Injected digital synovial sheath; 6. Lateral view of carpus; 7. Lateral view of carpus mesial section showing extent of bursae, vaginal sheathes and joint capsules; 8. Lateral view of carpus and tarsus vessels injected; 9. Anterior view of No. 8; 10. Lateral view of tarsus showing injection of synovial sheath of long extensor of digit; 11. Injected stifle (knee) joint.

C, Maceration specimen of skin at border of hoof.

30. *Development of primitive cardiac loop in the rabbit with special reference to the bulbo-ventricular cleft and the origin of the interventricular septum. Models and slides.* H. A. MURRAY, JR. (introduced by H. von W. Schulte), Columbia University.

31. *Modeling embryos on glass plates.* H. MURYAMA (introduced by C. R. Stockard,) Cornell Medical School, New York City.

32. *Wax reconstructions and stained preparations illustrating the fetal development of the mammary gland in the albino rat.* J. A. MYERS, Institute of Anatomy, University of Minnesota.

33. *Demonstration of models illustrating the morphogenesis of the human thyroid follicle. 2. Demonstration of models illustrating the early morphogenesis of the thyroid gland in *squalus acanthias*.* E. H. NORRIS (introduced by C. M. Jackson), Institute of Anatomy, University of Minnesota.

34. *Microscopic preparations showing various stages in the developmental changes of the idiosome.* GEORGE N. PAPANICOLAOU, Cornell Medical School, New York City.

35. *Specimens illustrating the histology of proœstrous and ovulation in the guinea-pig.* GEORGE N. PAPANICOLACU, Cornell Medical School, New York City.
36. *Sections and dissections of the notochord of an East Indian scorpion.* WILLIAM PATTEN, Dartmouth College.
37. *Models of the urogenital tract of the 20 mm. pig embryo.* FRANK H. ROSE (introduced by Franklin P. Johnson), Harvard Medical School.
38. *On the development of the first head-vein and its relation to the so-called vena capitis media and vena capitis lateralis.* FLORENCE R. SABIN, Anatomical Department, Johns Hopkins University.
39. *The early stages of the development of the great veins and of the hepatic circulation in the cat. Models and slides.* H. VON W. SCHULTE, Columbia University.
40. *Reconstructions showing developing nerves and arteries in the lower extremity, man and pig.* H. D. SENIOR, University and Bellevue Hospital Medical College.
41. *Specimens of hypophysectomized and control tadpoles (*Rana boylei*).* P. E. SMITH, University of California.
42. *The intracentral course of efferent cranial nerve roots traced by the method of indirect Wallerian degeneration.* SUTHERLAND SIMPSON, Cornell University Medical College, Ithaca.
43. *Breeding cages, inhalation cages and tanks, and a colony of mammals having been experimentally used for different periods up to six years in a study of the production and inheritance of morphological abnormalities.* C. R. STOCKARD, Cornell Medical School, New York City.
44. *Drawings illustrating the anatomy of the albatross.* R. M. STRONG, Anatomical Laboratories, Vanderbilt University Medical School.
45. *Preparations showing the mitochondrial content of the cells of the nuclei of the cranial nerves of the albino rat.* MADGE DE G. THURLOW, Johns Hopkins University and the Wistar Institute of Anatomy (introduced by E. V. Cowdry).
46. 1. *The generating substratum of the 'Membrana tectoria' represented by the crista spiralis and the epithelial ridges.* 2. *Genesis of the teeth of Huschke.* 3. *The teeth of Huschke in the adult organ.* 4. *Stages of development of the membrana reticularis at the surface of the auditory epithelium: Crista and Macula Acoustica and organ of corti.* O. VAN DER STRICHT (by invitation), Western Reserve University.

47. *Models of the roof of the fore brain in early human embryos.* JOHN WARREN, Harvard Medical School.
48. *Models of the coronary vessels in the pig embryo.* T. F. WHEELDON (introduced by F. P. Johnson,) Harvard Medical School.
49. (1) *Dissection and drawings of a human trunk showing situs totalis viscerum inversus;* (2) *Drawings and lantern slides from a dissection showing anomalous origin of (a) Arteria Gastrica Sinistra, (b) Arteria Mesenterica Superior, and (c) Arteria Spermaticae Internae in the same cadaver;* (3) *Drawing and lantern slides from a dissection showing (a) anomalous origin of Arteria Lienalis, (b) absence of Arteria Coeliaca, (c) anomalous origin of Arteria Gastrica Dextra, Arteria Gastrica Sinistra, Arteria Hepatica, in the same cadaver.* H. O. WHITE, Anatomical Laboratory of the College of Physicians and Surgeons, University of Southern California.
50. *Models of the heart and digestive tract in the 12 day 18 hour, and the 14 day albino rat embryos.* CHESTER H. HEUSER, The Wistar Institute.

CONSTITUTION

ARTICLE I

Section 1. The name of the Society shall be "The American Association of Anatomists."

Sec. 2. The purpose of the Association shall be the advancement of anatomical science.

ARTICLE II

Section 1. The officers of the Association shall consist of a President, a Vice-President, and a Secretary, who shall also act as Treasurer. The President and the Vice-President shall be elected for two years, the Secretary for four years. In case of absence of the President and Vice-President, the senior member of the Executive Committee shall preside. The election of all the officers shall be by ballot at the annual meeting of the Association and their official term shall commence with the close of the annual meeting.

Sec. 2. At the annual meeting next preceding an election, the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual meeting. Additional names for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE III

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of eleven members, including the officers. Two members of the Executive Committee shall be elected annually and, so far as possible, election of members of the Executive Committee shall be in proportion to the geographical distribution of members. Five shall constitute a quorum of the Executive Committee.

ARTICLE IV

The Association shall meet at least annually, the time and place to be determined by the Executive Committee. The annual meeting for the election of officers shall be the meeting of convocation week, or in case this is not held, the first meeting after the new year.

ARTICLE V

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences, and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recom-

mendation by a list of the candidate's publications, together with references. Their election by the Executive Committee, to be effective, shall be ratified by the Association in open meeting.

Sec. 2. Honorary members may be elected from those who have distinguished themselves in anatomical research. Nominations by the Executive Committee must be unanimous and their proposal with a reason for recommendations shall be presented to the Association at an annual meeting, a three-fourths vote of members present being necessary for an election.

ARTICLE VI

The annual dues shall be seven dollars. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated at the discretion of the Executive Committee on payment of arrears.

ARTICLE VII

Section 1. Twenty members shall constitute a quorum for the transaction of business.

Sec. 2. Any change in the constitution of the Association must be presented in writing at one annual meeting in order to receive consideration and be acted upon at the next annual meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

Sec. 3. The ruling of the Chairman shall be in accordance with "Robert's Rules of Order."

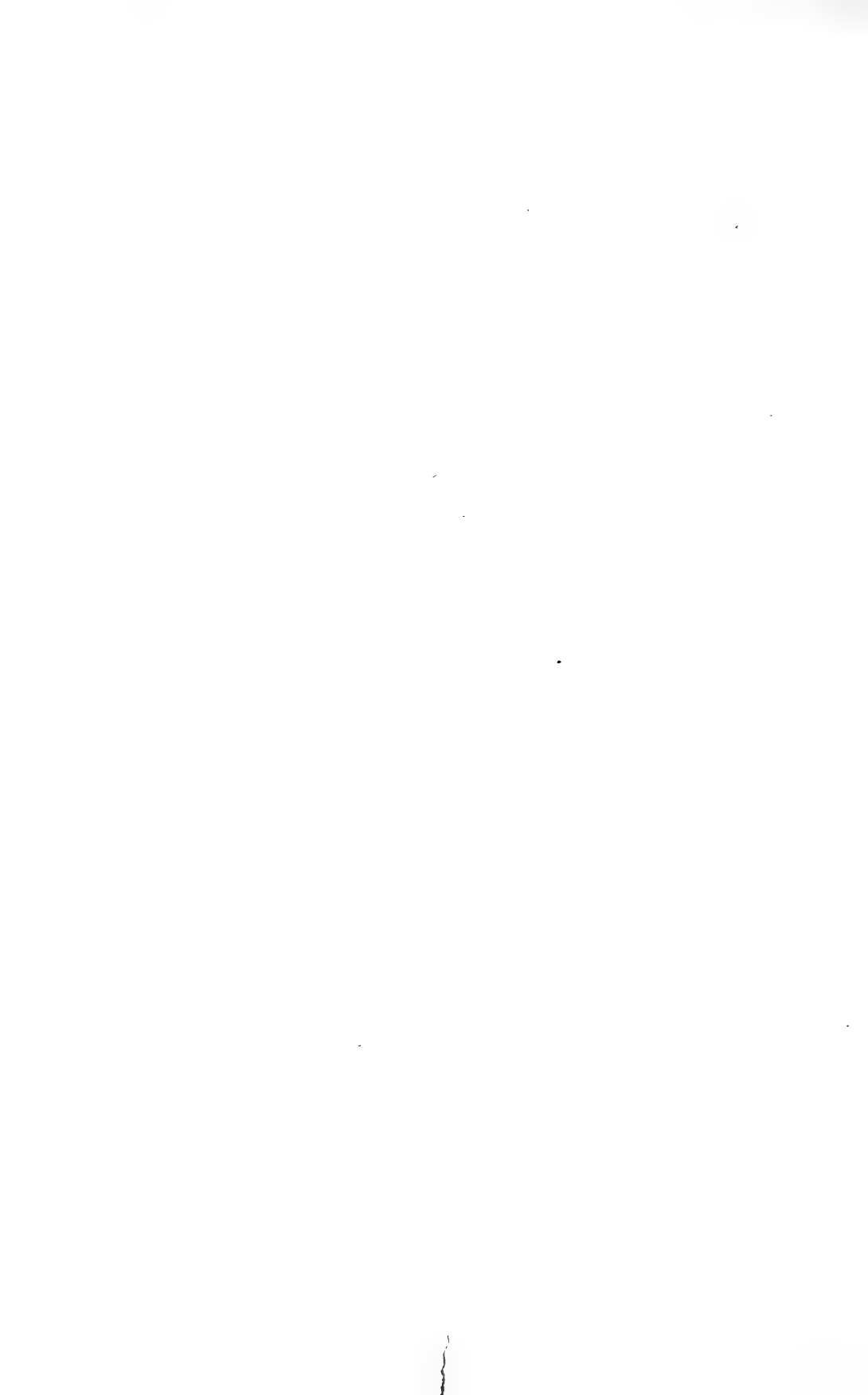
The orders adopted by this Association, which read as follows, have not been altered:

Newly elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be fifteen minutes.

The Secretary and Treasurer shall be allowed his traveling expenses and the sum of \$10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings and that the Anatomical Record be sent to each member of the Association, on payment of the Annual Dues, this journal to publish the proceedings of the Association.



AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

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Vice-President.....CLARENCE M. JACKSON
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 For term expiring 1918.....HERMANN VON W. SCHULTE, JOHN L. BREMER
 For term expiring 1919.....ELIOT R. CLARK, REUBEN M. STRONG
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Committee on International Congress

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PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOOLOGISTS

FOURTEENTH ANNUAL MEETING

The American Society of Zoologists held its fourteenth Annual Meeting jointly with Section F of the American Association for the Advancement of Science and in affiliation with the American Society of Naturalists, December 27, 28 and 29, 1916, in Schermerhorn Hall, Columbia University, New York City.

BUSINESS SESSION

Election of Members

At the session for transacting business, held at 11 o'clock on Friday, December 29, President D. H. Tennent in the chair, the persons whose names follow, having been recommended by the Executive Committee to the Society for election to membership, we duly elected.

- ALLEN, EZRA, A.M., Ph.D. (University of Pennsylvania), Professor of Biology, *Philadelphia School of Pedagogy, 12th above Spring Garden, Philadelphia, Pa.*
- CAROTHERS, E. ELEANOR, A.B., A.M., Ph.D., *Zoological Building, University of Pennsylvania, Philadelphia, Pa.*
- CHURCHILL, EDWARD PERRY, A.B. (Iowa), Ph.D. (Johns Hopkins), Assistant U. S. Bureau of Fisheries, *317 Marshall Street, Hampton, Va.*
- CORT, WILLIAM WALTER, A.B. (Colorado College), M.A., Ph.D., University of Illinois, Assistant Professor of Zoology, University of California, *Department of Zoology, University of California, Berkeley, Calif.*
- DOLLEY, JR., WILLIAM LEE, A.B., A.M. (Randolph-Macon), Ph.D. (Johns Hopkins), Professor of Biology, *Randolph-Macon College, Ashland, Va.*
- GEE, WILSON, B.S. (University of S. C.), Ph.D. (University of California), Professor of Biology, *Emory University, Oxford, Ga.*
- GOODRICH, HUBERT BAKER, B.S. (Amherst), M.A., Ph.D. (Columbia), Instructor in Zoology, *Wesleyan University, Middletown, Conn.*
- HALL, MAURICE CROWTHER, S.B., M.A., Ph.D., D.V.M., Parasitologist, *Research Laboratory, Parke, Davis & Co., Detroit, Mich.*
- IBSEN, HEMAN LAWRTITZ, B.S., M.D., Ph.D. (Wisconsin), Assistant in Experimental Breeding, *University of Wisconsin, Madison, Wis.*

- JONES, ORREN LLOYD, B.S., M.S., Ph.D. (Wisconsin), Associate Professor, Animal Husbandry, *Iowa State College, Ames, Iowa.*
- KINCAID, TREVOR, M.S., Professor of Zoology, *University of Washington, Seattle, Wash.*
- METZ, CHARLES W., B.A., Ph.D., Station for Experimental Evolution, *Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N. Y.*
- MIDDLETON, AUSTIN RALPH, A.B., Ph.D. (Johns Hopkins), Assistant Professor of Biology, *University of Louisville, Louisville, Ky.*
- MOSHER, EDNA, B.S. (Cornell), Ph.D. (Illinois), Instructor in Entomology, *University of Illinois, Natural History Building, Urbana, Ill.*
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- WENTWORTH, EDWARD N., M.S. (Iowa), Professor of Animal Husbandry, *Kansas State Agricultural College, Manhattan, Kans.*
- WHITING, PHINEAS W., A.B., M.S., Ph.D., Harrison Research Fellow, *University of Pennsylvania, Zoological Laboratory, Philadelphia, Pa.*

Election of Officers

The Committee on Nominations, consisting of E. A. Andrews, Edwin Linton and C. A. Kofoed, having recommended persons for election to the various offices of the Society, and no other nominations having been made, Maynard Mayo Metcalf was elected President to serve for one year, Charles Zeleny, Vice-President to serve for one year, Caswell Grave, Secretary-Treasurer to serve three years, and H. V. Wilson, Member at large of the Executive Committee to serve five years.

Report of the Secretary-Treasurer

The Secretary-Treasurer reported that 40 members withdrew from the Society during the year and that 26 members have not paid dues, in protest against the increase in annual dues, but that

257 members have accepted the plan, adopted at the Columbus meeting, for supporting and increasing the circulation of Journals published by The Wistar Institute. Attention was called to the fact that the list of members of the Society now contains 308 names of which 173 (56 per cent) are of members residing in Eastern territory, 135 (44 per cent) are of members residing in Western territory.

Financial Statement

The financial statement of the Secretary-Treasurer, for the year 1916, is as follows:

RECEIPTS

January 1, 1916:	
Balance on hand.....	809.68
Back dues for the year 1913.....	2.00
Back dues for the year 1914.....	8.00
Back dues for the year 1915.....	36.00
January to December:	
Dues for 1916 at \$11.50.....	126.50
Dues for 1916 at 8.00 (Foreign).....	8.00
Dues for 1916 at 7.00.....	1225.10
Dues for 1916 at 6.50 (Life Members).....	19.50
Dues for 1916 at 6.00.....	6.00
Dues for 1916 at 5.00.....	335.10
October 1, 1916:	
Interest at 4 per cent on deposits.....	32.61
Total.....	\$2608.49

EXPENDITURES

January to December:	
Appropriation for Councilium Bibliographicum.....	200.00
Express charges.....	4.35
Telegraph, telephone and street car.....	2.60
Two copies "Science" containing Proceedings.....	.30
Stationery, stamps, post-cards.....	32.82
Typewriting and clerical assistance.....	31.65
Printing announcements and programs.....	51.55
Overpaid dues returned to H. F. O.....	5.00
257 subscriptions for Journals, Wistar Inst.	1591.00
Expenses of Secretary at Conference on Journals.	4.80
Expenses of Secretary at New York meeting.....	34.84
Total.....	\$1958.91
December 28, 1916:	
Balance on hand.....	\$649.58

Report of Auditing Committee

The Auditing Committee, consisting of Ulric Dahlgren and William H. Longley, reported that the accounts of the Secretary-Treasurer had been examined and found correct.

Instruction for Executive Committee

By motion made by P. P. Calvert, seconded by C. E. McClung, the Society instructed its Executive Committee to continue to make efforts to increase the number and variety of Journals available to members in return for annual dues.

Change in By-Laws

By vote of the Society, by-law 4 b, which reads as follows; was stricken from the list of by-laws.

4(b) When the annual meeting is held in conjunction with the American Society of Naturalists, the Society shall adjourn for one session (morning or afternoon) to meet in joint session with the American Society of Naturalists, and it shall be the policy of the Society to conclude its annual meeting with the Naturalists' dinner, and the Secretary-Treasurer is instructed to urge upon the Naturalists the propriety of adjusting their program with this point in view.

Resolution Adopted

E. G. Conklin proposed the following resolution which was unanimously adopted by the Society:

"Whereas, the National Academy of Sciences has, at the request of the President of the United States, organized the National Research Council for the purpose of promoting and organizing research in the interest of National Welfare, and

"Whereas, we recognize that human progress is dependent upon the advancement of knowledge, and

"Whereas, one of the chief purposes of the American Society of Zoologists is the promotion of research in Zoology,

"Therefore, be it resolved that the American Society of Zoologists agrees to cooperate with the National Research Council in any ways practicable."

Committee on Premedical Education

The Committee on Premedical Education, failing again to submit a report, was continued and instructed to report at a future meeting.

JOINT SESSION WITH NATURALISTS

The business of the Society having been transacted, the session adjourned to meet in joint session with the American Society of Naturalists, at 2 p.m., in the Horace Mann School, the program for which was a symposium on the subject, "Biology and National Existence."

Sessions for Presentation and Discussion of Papers

At sessions held during the forenoons and afternoons of December 27 and 28 and during the first part of the forenoon session on December 29, the papers listed on the program were read in full or by title. Forty-four papers were presented in full, twenty-seven were read by title. The papers listed in the Genetics Section, as ordered by the Society at the Columbus Meeting, were scheduled for and read at the session on the afternoon of December 28.

TITLES OF PAPERS

LIST OF TITLES OF PAPERS ARRANGED IN GROUPS IN THE ORDER RECEIVED BY THE SECRETARY

COMPARATIVE ANATOMY

1. Cell inconstancy in *Hydatina senta*. A. Franklin Shull, University of Michigan.
2. A gynandromorphous cat. Mary T. Harmon, Kansas State Agricultural College.
3. On the third layer of protoplasm in amoeba. A. A. Schaeffer, University of Tennessee.
4. Some homologies in the epipharynx and hypopharynx of the nematoceros diptera. Adelbert L. Leathers, Olivet College (Section F).

EMBRYOLOGY

5. The history of the eye muscles. (Lantern illustrations.) H. V. Neal, Tufts College.
6. A case of superfetation in the cat. Mary T. Harmon, Kansas State Agricultural College.
7. On the mechanism of serial differentiation in the embryonic vertebrate nervous system. O. C. Glaser, University of Michigan.
8. Embryology of the yellow mouse. W. B. Kirkham, Yale University.
9. Investigations of the light organs of arthropods. Ulrich Dahlgren, Princeton University.
10. Further experiments on the laterality of transplanted limbs. Ross G. Harrison, Yale University.
11. The effect of removal and regeneration of parts upon metamorphosis in amphibian larvae. Charles Zeleny, University of Illinois.
12. Life history of *Zeugophora scutellaris*. B. H. Grave, Knox College.
13. The results of extirpation of the hypophysis and thyroid glands of *Rana*. Bennet M. Allen, University of Kansas.

CYTOLOGY

14. An experimental study of cell division. L. V. Heilbrun, University of Illinois College of Medicine.
15. Early castration of the vertebrate embryo. Franklin P. Reagan, Princeton University. Introduced by C. F. W. McClure.
16. Microdissection studies. The cell aster; a reversible gelation phenomenon.— Illustrated with drawings. Robert Chambers, Jr. Cornell University Medical College.
17. Multiple chromosomes of *Hesperotettix* and *Mermiria*. C. E. McClung, University of Pennsylvania.
18. The spermatogenesis of *Culex pipiens*, P. W. Whiting, University of Pennsylvania. (Section F.)
19. The segregation and recombination of homologous chromosomes in two genera of Acrididae (Orthoptera). E. Eleanor Carothers, University of Pennsylvania. (Section F.)
20. Synapsis and chromosome organization in the male germ cells of *Chortippus* and *Trimerotropis*. D. H. Wenrich, University of Pennsylvania. (Section F.)
21. The chromosome complex in *Apithes agitator*. W. J. Baumgartner, University of Kansas.

22. New facts and views concerning the occurrence of a sexual process in the life cycle of a myxosporidia, *chloromyxum leydigi*. Rhoda Erdmann, Yale University.
23. Spermatogenesis in the albino rat. Ezra Allen, University of Pennsylvania. (Section F).
24. Multiple complexes in the alimentary tract of *Culex pipiens*. Caroline M. Holt, University of Pennsylvania. (Section F).

GENETICS

25. Sex-linked inheritance of spangling in poultry. George Lefevre, University of Missouri.
26. Two classes of factors for color patterns in *Paratettix*. Robert K. Nabours, Kansas State Agricultural College.
27. The relation of yellow coat color to black eyed white spotting of mice, in heredity. C. C. Little, Harvard Medical School.
28. Mutation in *Didinium nasutum*. S. O. Mast, Johns Hopkins University.
29. The occurrence of mutations in skunks of the species, *Mephitis putida* and *M. hudsonica*. J. A. Detlefsen, University of Illinois, College of Agriculture.
30. The influence of parental alcoholism on the learning capacity of the offspring. E. C. MacDowell, Carnegie Institution of Washington.
31. Linkage in the sex-chromosome of a new species of *Drosophila*. Chas. W. Metz, Carnegie Institution of Washington. Introduced by C. B. Davenport.
32. An examination of the so-called process of contamination of Genes. Thomas Hunt Morgan, Columbia University.
33. An analysis of the effect of selection on bristle number in a mutant race of *Drosophila*. Alfred H. Sturtevant, Columbia University.
34. The elimination of males in alternate generations of sex-controlled lines. Calvin W. Bridges, Columbia University. Introduced by T. H. Morgan.
35. Coincidence of crossing over and the chromosome theory of linkage. Alexander Weinstein, Columbia University. Introduced by T. H. Morgan.
36. Determinate and indeterminate laying cycles in birds. Illustrated with lantern. L. J. Cole, University of Wisconsin.
37. A strain of sex intergrades. Arthur M. Banta, Carnegie Institution of Washington.
38. Effect on fertility of crossing closely and distantly related stocks of *Drosophila ampelophila*. Roscoe R. Hyde, Indiana State Normal School.
39. Are the polyradiate cestodes mutations? Illustrated with lantern. Franklin D. Barker, University of Nebraska.

EVOLUTION

40. A revised working hypothesis of mimicry. W. H. Longley, Goucher College.

COMPARATIVE AND GENERAL PHYSIOLOGY

41. Recent studies of nerve conduction in *Cassiopea*. Illustrated with lantern. Alfred G. Mayer, (Carnegie Institution of Washington).
42. The theory of sex as stated in terms of results of studies on the pigeons. Oscar Riddle, Carnegie Institution of Washington.
43. The adaptive color changes of tropical fishes. Illustrated with lantern. W. H. Longley, Goucher College.
44. The histological basis of adaptive shades and colors in the flounder, *Paralichthys albiguttus*. Albert Kuntz, St. Louis University School of Medicine.
45. Further data on the relation between the gonads and the soma of some domestic birds. H. D. Goodale, Massachusetts Agricultural Experiment Station.
46. The sensory potentialities of the nudibranch *Rhinophores*. Leslie B. Arey, Northwestern University Medical School.

47. *Paramecium* grown in pure cultures of bacteria. George T. Hargitt and Walter W. Fray, Syracuse University.
48. Recognition among insects. N. E. McIndoo, Bureau of Entomology.
49. The rate of locomotion of *Vanessa antiopa* in different luminous intensities and its bearing on the "Continuous Action theory of Orientation." Wm. L. Dolley, Jr., Randolph-Macon College. Introduced by S. O. Mast.
50. A super-organ for the expansion of *Renilla*. G. H. Parker, Harvard University.
51. The photoreceptors of *Amphioxus*. W. J. Crozier, Bermuda Biological Station.
52. The olfactory reactions of snails. Manton Copeland, Bowdoin College.
53. The reactions of the crimson-spotted newt, *Diemetylus viridescens* to light. Albert M. Reese, West Virginia University.
54. Reaction of the whip-tail scorpion to light. Bradley M. Patten, Western Reserve University.
55. The effect of light and dark upon the eye of *prorhynchus applanatus*, Kennel. W. A. Kepner, and A. M. Foshee, University of Virginia.
56. Experimental control of endomixis in *Paramecium*. R. T. Young, University of North Dakota.
57. Orientation to light in *Planaria* (n. sp.) and the function of the eyes. W. H. Taliaferro, Johns Hopkins University. Introduced by S. O. Mast.
58. Sense of taste in *Nereis virens*. Alfred O. Gross, Bowdoin College.
59. The Influence of the Marginal Sense Organs on Functional Activity in *Cassiopea xamachana*, Bigelow. Lewis R. Cary, Princeton University.
60. The relation between the hydrogen ion concentration of sperm suspensions and their fertilizing power. Edwin J. Cohn, University of Chicago. Introduced by Frank R. Lillie.
61. Experimental study of ageing eggs and sperm and of their development. A. J. Goldfarb, College of the City of New York.
62. The consumption of oxygen during the development of *Fundulus heteroclitus*. George G. Scott, College of the City of New York, and William E. Kellicott, Goucher College.
63. A study of broodiness in the Rhode Island Red breed of domestic fowl. H. D. Goodale, Massachusetts Agricultural Experiment Station.
64. The vitality of cysts of *Didinium nasutum*. S. O. Mast, Johns Hopkins University.
65. The reactions of *Pelomyxa Carolinensis*, Wilson, to food. W. A. Kepner and J. G. Edwards, University of Virginia.
66. The significance of conjugation and encystment in *Didinium nasutum*. S. O. Mast, Johns Hopkins University.

ECOLOGY

67. Some distributional problems of Okefinokee Swamp. A. H. Wright, Cornell University.

PARASITOLOGY

68. A means of transmitting the fowl nematode, *Heterakis papillosa* (Bloch). James E. Ackert, Kansas State Agricultural College.
69. Further studies on changes in *Thelia bimaculata* brought about by insect parasites. Illustrated with lantern. S. I. Kornhauser, Northwestern University.
70. Some experiments on the transmission of swamp fever by insects. Illustrated with lantern. John W. Scott, University of Wyoming.
71. The domestic cat a host of *taenia pisiformis* (Bloch). James E. Ackert, Kansas State Agricultural College.

DEMONSTRATIONS

1. Preparations showing the structure of a transformed plasma Clot. George A. Baitsell, Yale University.

2. Demonstrations of the following types of chromosome groups in *Drosophila ampelophila*, XX (female), XY (male), XXY (female), XXYY (female). Calvin W. Bridges, Columbia University.
3. The innervation of the vertebrate digestive tube (Methylene blue intravital staining) F. W. Carpenter, Trinity College.
4. The phosphorescence of enteropneusta. W. J. Crozier, Bermuda Biological Station.
5. The relation between the gonads and the secondary sexual characters in birds. H. D. Goodale, Massachusetts Agricultural Experiment Station.
3. Mounted skins showing a new color variety of the Norway rat. Helen D. King and P. W. Whiting, University of Pennsylvania.
7. Photographs of *Thelia* illustrating the changes brought about by the parasitic hymenopteron *Aphelopus*. S. I. Kornhauser, Northwestern University.
8. Multiple chromosomes of *Hesperotettix* and *Mermiria*. C. E. McClung, University of Pennsylvania.
9. Models and specimens showing transplanted limbs. Ross G. Harrison, Yale University.
10. Microscopic slides illustrating spermatogenesis in *Culex pipiens*. P. W. Whiting, University of Pennsylvania. (Section F).
11. Specimens of Polyradiate cestodes. Franklin D. Barker, University of Nebraska.

1. *Cell inconstancy in Hydatina senta*. A. FRANKLIN SHULL, University of Michigan.

The number of cells in the various organs of this rotifer was reported by Martini to be always the same. In at least two organs, however, it is found that in a small percentage of individuals aberrant numbers occur. This inconstancy is more in keeping with the well established variability and modifiability of the life cycle.

2. *A Gynandromorphous cat*. MARY T. HARMON, Kansas State Agricultural College.

A cat having an ovary on the right side and a testis on the left, was discovered in our laboratories last winter. The animal had been skinned and partially dissected before its peculiarity was discovered. The scrotal sac and external genitalia had been removed.

The testis which is about the size and shape of a navy bean is entirely on the outside of the body cavity ventrad and to the left of the ventral border of the pubis. It has all the appearance of a normal testis. The spermatic cord extends from the testis through the oblique muscle where it divides into the vas deferens and the spermatic vein and artery. The vas deferens extends anteriorly until it curves over the ureter where it continues caudad dorsal to the neck of the urinary bladder. It pierces the prostate gland and enters the urethra about half way between the base of the urinary bladder and the exterior. The prostate gland of the left side is larger than the one of the right side although there seems to be a gland on the right side.

The ovary is located on the right side of the body a little posterior to the kidney. It is slightly smaller than the testis and is quite angular. Anterior to the ovary and partially surrounding it is the ostium tubae abdominale. The ovarian artery and the ovarian vein extend to the left from the ovary. Extending from the ostium tubae abdomi-

nale is the uterine tube which continues caudad almost parallel to the vas deferens. It enters the urethra through the abortive right prostate gland. The ovary and the uterine tube are held in place by the broad ligament and the round ligament.

3. *On the third layer of protoplasm in ameba.* A. A. SCHAEFFER, University of Tennessee.

In addition to the streaming endoplasm and the more or less stationary ectoplasm in amebas there is found a third layer separating the ectoplasm from the surrounding water. This third layer is extremely thin, probably too thin to be seen easily, but the existence of it can be readily demonstrated by means of the movement of small particles which occasionally attach themselves to it. The most striking feature of the movement of particles attached to this third layer is that *they move forward toward the tip of a pseudopod faster than the tip advances through the water.* Particles travel from every part of the surface toward the tips of the advancing pseudopods. If there is but one pseudopod, all the particles travel toward the tip of this pseudopod. The effect is therefore that all the particles tend to collect at the advancing end of the ameba. Most of the particles drop off however when or soon after they reach the front end of a pseudopod. Particles travel at varying speeds, depending upon where they are located; particles on an actively enlarging pseudopod move faster than those near the posterior end of the ameba. In a general way, the nearer they approach the tip of a pseudopod the faster the particles travel. The speed of a moving particle attached to the third layer is not directly affected by nor related to the rate of streaming of the endoplasm immediately under it (but separated from it, of course, by the ectoplasm). The granules in the streaming endoplasm of a pseudopod move considerably faster than the particles attached to the outside third layer. But occasionally one may see a particle sticking to the third layer move more rapidly than the slowly and uncertainly moving endoplasm immediately beneath it. When streaming is reversed in a pseudopod, movement in the third layer is likewise reversed, and frequently particles attached to the outside travel away from the tip of the retracting pseudopod more rapidly than the tip is retracted. These particles then move toward the tip of another advancing pseudopod. Occasionally a particle sticks so close that it travels all over an ameba before it finally drops off. This third layer is found in *Amoeba proteus*, *A. discoides*, *A. dubia*, *A. vespertilio*, and *A. metaproteus* (an undescribed species).

Whatever may be the relation of this third layer to endoplasmic streaming, it is clear that from the transportative aspect it is not an aid but a slight hindrance to locomotion, since the layer moves in the same direction as the ameba.

The nature of this layer is not clear. It seems to be in process of continual formation all over the ameba, and in process of continual destruction at the anterior ends of pseudopods. Surface is not therefore made at the advancing tips of the pseudopods, as might be thought

at first sight, but destroyed, i.e., converted into interior (non-surface) matter. Surface is made over the entire ameba, but very slowly at the posterior end and at the tips of retracting pseudopods. From inspection it appears that the layer is protoplasmic in composition rather than aqueous, though there is insufficient evidence at hand to decide definitely. It is also at this time impossible to state whether there is essential connection between locomotion and the movement of this third layer.

The pseudopods of *Diffugia pyriformis* carry foreign particles on their surfaces, but these particles do not move as rapidly as the tips of the pseudopods advance. Diatoms and *Oscillatoria* likewise carry particles on their surfaces, but in the latter the same agency that carries the particles also moves the *Oscillatoria* filaments. Whether the transportative agencies of these several organisms have anything in common cannot yet be stated.

4. *Some homologies in the epipharynx and hypopharynx of the nematoceros diptera.* ADELBERT L. LEATHERS, Olivet College, Section F.

The literature bearing on this subject is very incomplete and more or less confused. The 'lateral arms' or 'premandibles' of the hypopharynx have been well figured for only the Chironominae, while in the other families and subfamilies of the group no such structures have hitherto been recognized. These structures will be shown by a series of comparative figures with especial reference to the family Chironomidae, but will include one or more other families in less detail. In a similar manner the comparative development of the hypopharynx from a rudimentary structure to a highly developed triturating organ will be compared.

5. *The history of the eye muscles.* H. V. NEAL, Tufts College. (With lantern illustrations.)

The attempt is made in this paper to demonstrate on the basis of embryological evidence the exact homology of the first three permanent myotomes of *Amphioxus*, *Petromyzon*, and *Squalus* and to describe the more important stages in the phylogenesis of the eye muscles.

The evidence is presented for the first time to support the assertion of Dohrn ('04) and the writer ('07) that the second as well as the third myotome participates in the formation of the external rectus muscle. In the light of the evidence given the familiar text-book formula for the ontogenesis of the eye muscles should be amended as follows:

From the first myotome (pre-mandibular head-cavity) arise the muscles innervated by the oculomotor, viz., the Mm. recti superior, internus, and inferior, and the M. obliquus inferior;

From the second myotome (mandibular head-cavity) develop the M. obliquus superior and the ventro-lateral portion of the M. rectus externus;

From the third myotome (hyoid head-cavity) arises the dorso-medial portion of the M. rectus externus.

6. *A case of superfetation in the cat.* MARY T. HARMAN, Kansas State Agricultural College.

The gravid uterus of a cat had two enlargements of the right horn and three enlargements of the left horn. The enlargement of the right horn next to the ovary and the two enlargements of the left horn toward the ovary each contained an embryo 90 mm. in length exclusive of the tail. From the external features, they appear to be near to term. The limbs are well formed and normal, having joints and on the ends of the digits are claws. The whole surface of the skin is covered with pits but very little hair is present. The tail is more than one-third the length of the body. The fetal membranes fill the entire enlargement and fit very closely to its walls.

The enlargement of the right horn of the uterus next to the vagina contained an embryo only 10 mm. in length which seemed perfectly normal and which had no indications of having been dead long before it was preserved. The umbilical cord occupies about one-sixth of the ventral surface. The limbs both fore and hind, are merely buds. The tail is about one-fifth the length of the remainder of the body. There are no indications of hair. The mouth is in the process of formation. The mandibular processes have met in the median line; but they extend only about one-third as far as the maxillary processes. The lip groove is shallow, in fact, there is merely the beginning of the separation of the lips and cheeks from the jaw. The oral pit is rectangular in shape. There is no indication of eyelids; but the eyes are plainly visible from the outside as small dark spheres. This embryo does not seem to be of more than two weeks development.

If the size of the blood vessels of the uterus and the condition of the blood vessels to the embryos may be taken as a criterion, the blood supply to the small embryo is as good as to the large embryos. It seems as reasonable to the writer to think of the less advanced embryo as the result of delayed fertilization as to account for it on the ground of arrested development or of a second coition.

7. *On the mechanism of serial differentiation in the embryonic vertebrate nervous system.* O. C. GLASER, University of Michigan.

Long before the separation of the neural plate from the ectoderm is complete, the developing neural tube prefigures a serial differentiation that culminates in a succession of vesicles highly constant for the vertebrate nervous system and, as one of its basic attributes, calling for explanation.

More than forty years ago His attempted to account for the vesiculation of the embryonic brain in terms of cranial flexure. Substantiated by ingenious experiments with rubber models, this theory could account for the early lateral and ventral differentiations of the prospective interbrain. However, since the onset of vesiculation occurs before that of cranial flexure the view that the former is dependent upon the latter involves an anachronism.

According to His cranial flexure and the subsequent rearrangement of the vesicles are the outcome of differential growth which produces a state of compression in the antero-posterior axis. It is obvious that such compression, if demonstrable prior to cranial flexure, would necessarily be an important element in helping us to understand how the nervous system has imposed upon it its characteristic form.

Compression in the antero-posterior axis cannot be demonstrated by the method used by His. It can, however, be shown to exist by comparing the length of the embryonic head with that of the nervous system. The relation between these two measurements can be expressed in the form of a fraction derived by dividing half the perimeter of the nervous system into the head length. This fraction, which I have called the neurocephalic quotient, tells how many units of head-length are available for every unit of length in the nervous system.

When the quotient is greater than unity, the nervous system cannot be under compression; when it is less than unity, a state of compression must exist. A priori we should expect high quotients in the earlier stages and low quotients in the later, whereas embryos in which the vesicles are telescoped or abnormally overdifferentiated, should have quotients lower than normal for their respective ages.

These expectations, as will be shown in the paper, are fulfilled, and the conclusion seems warranted that a rising state of compression in the longitudinal axis, prior to cranial flexure, is one of the important conditions under which the vesiculation of the embryonic brain takes place.

The paper will be fully illustrated by tables, drawings, and a curve.

8. *Embryology of the yellow mouse.* W. B. KIRKHAM, Osborn Zoological Laboratory, Yale University.

It has for some years been known to breeders that yellow mice did not breed true to coat color, each litter almost always containing one or more young of a color other than yellow, and it has also been found that the average number of young in a litter is smaller from yellow than from other colors of mice. These two observed facts taken together have given rise to the theory that all the available yellow mice are heterozygous as regards coat color, and that the homozygous zygotes which should theoretically exist are for some reason not viable. To test out this theory has been the purpose of this work.

Material from non-suckling yellow mice, representing each of the first nineteen days of pregnancy has been assembled, that for the first three days comprising ovaries and Fallopian tubes, that for stages beyond the third day the uteri as well as the ovaries and tubes. The entire material from each mouse has been sectioned, mounted, stained, and every section examined, while all the embryonic stages found have been compared with those of like age from non-suckling white mice. The results are as follows:

(a) The rate of cleavage and of embryonic development is the same for yellow as for white mice.

(b) All of the observed two-cell stages of both yellow and white mice appear normal.

(c) No degenerating morulae or blastulae were found in white mice, while one or more were present in every yellow mouse containing embryos of that stage in development.

(d) The material covering the sixth to the seventeenth days of pregnancy has yielded degenerating embryos in eight uteri out of twenty-eight in white mice, and in eleven out of thirteen in yellow mice. If we eliminate females who, by having still births or by eating up their new-born young, showed themselves abnormal, the figures become more striking, degenerating embryos in white mice appearing only in one uterus out of the twelve examined, while in yellow mouse uteri eleven out of twelve contained them.

(e) After the normal time for implantation (sixth day of pregnancy) all the degenerating embryos found, with one exception, had induced a typical reaction on the part of the uterine mucosa, but had themselves failed to undergo any development beyond the blastula stage.

(f) No degenerating embryos have been found in either white or yellow mice pregnant more than sixteen days. This is to be expected, as the evidence all indicates that if degeneration is going to take place it starts before the seventh day of pregnancy, and the embryos are then so small that the phagocytes easily remove them, and the uterine wall in their vicinity returns to its resting condition several days before the normal embryos are born.

(g) Mendel's law requires an average of 25 per cent homozygous yellow offspring from heterozygous yellow parents. In this investigation we have found in normal yellow females, six to seventeen days pregnant, 69 embryos, of which 26 or 37.8 per cent were degenerating, as contrasted with 2 degenerating embryos out of a total of 84, or 2.3 per cent obtained from normal white females pregnant for the same period of time.

Conclusion. The evidence produced by this investigation is not as absolutely decisive as might be hoped for, but the much greater percentage of degenerating embryos in yellow mice than in white would indicate that some at least of these degenerating yellow mouse embryos are the missing homozygous animals.

9. *Investigations of the light organs of arthropods.* ULRICH DAHLGREN, Princeton University.

The origin of the imaginal light organs in *Photurus pennsylvanica* was investigated to find out how it was derived during the pupal stage. Larvae, bearing their two round ventro-lateral organs on the penultimate segment, were collected in the spring and placed in vivaria containing soil and dead leaves from their habitat. In latter May these larvae scooped shallow cavities in the ground, built lattice-shaped covers with a mixture of saliva and clay and became torpid. In a few days the skin was shed and the cream-white pupae lay in the cavities when they could be easily observed after breaking off the clay covers and

placing loose leaves over the nest. Eight to eleven days at the prevailing temperature saw them change into the adult fly. During this time a series of successive browning and blackening of different parts of the underlying integument of the imago marked their gradual development and gave a series of marks to establish a correct chronology upon which to establish the successive stages of the metamorphosis of the light organs. At first only the two larval organs showed. They were always ready to light up on stimulation until about half way through the change when they lost the power for about twenty-four hours. At about this time the adult organs began to appear as whitened surfaces of the same segment that contained the larval light organ and also that segment immediately anterior to it. At about the sixth or seventh day this new surface began to light in a central spindle-shaped area in each side of the two segments and the lighting power spread out from these focii until shortly before hatching when the entire surfaces would light slowly if the pupae was disturbed in any way.

The internal changes accompanying this were briefly as follows; the small round larval organs began at an early date to be retired from their position against the cuticle and when the imaginal cuticle was formed the entire surface was lined by a hypodermis that showed no differentiation. Signs of histolysis became evident in the larval organ which had moved far in toward the center of the body. The new imaginal centers of the adult organs now showed a deposit of large, yolk-like granules in these cells. The new ventral hypodermis of the imaginal light segments also showed this accumulation of reserve food material as scattered pupal yolk globules.

Shortly afterward the hypodermis proliferated into several layers which grew rapidly into the light cells.

The origin of the reflector layer is somewhat obscure but appears to be a differentiation of the dorsal portion of these epithelial-derived cells. The trachae grow in and form the cylinders and the end cells appear just before hatching. The light organs of the Lampyridae are not developed from the fat organs as many authors have surmised but from the ventral integumental epithelium of the insect.

The luminous organs of the ostracod, *Cypridina hilgendorffii*, was also studied. Müller has already shown that light glands opened in several places on the upper lip of a closely allied, if not the same, form. Doflein undertook to study the organ more closely and was much at fault in his description. He described and drew a sac-shaped reservoir opening at the upper lip by several apertures. The luciferine was secreted by a large gland forming a part of the fundus of the sac. Doflein undoubtedly drew the brain or supra-esophageal ganglion for this gland as Yatsu pointed out to Dr. E. N. Harvey and as the writer concluded from reading the article and examining the drawing.

Studies of the organ in some beautifully preserved material from Japan collected for the writer by Dr. E. N. Harvey show the following conditions: The luminous organ consists of a group of from twenty to

thirty hypodermal cells invaginated from the edge of the upper lip into an elongate series of unicellular glands that reach up to and almost touch the upper brain ganglia. These cells are arranged bilaterally into two contiguous groups. They may be distinguished as of three different kinds by their secretion and form. The first two kinds secrete a series of granules that are basic in reaction, taking the acid dyes as eosin. One of these secretes a very large heavy weaker staining granule and the other a very small deeper staining granule. The cytoplasmic bodies of these cells form a compact rounded mass up near the brain. They show large single nuclei each with a very large central plasmosome.

The distal part of each cell reaches down to the median part of the upper lip and open through the chiten as a very short papillae. This distal region forms in each case a long hollow tube filled closely with the granules of the cell. It is possible that two cells sometimes open through a single papillae but it is not probable. The papillae are closely set together in a group.

The third kind of cells form a wide shallow common sac on each side of this median group. Their cytoplasmic bodies are united into the upper wall of this sac which serves as a common reservoir for the secretion. While shallower than in the case of the first cells, the cytoplasm of these third cells is of the same texture and holds the same kind of nucleus but its secretion is totally different, being much like mucous and staining the opposite (with the chromatin stains) of that of the first two kinds. There are two of these sacs and each opens through a very long slender papilla that hangs down laterally to the opening of the typical ostracod shell.

A peculiar point is that one of the coarse granular cells on each side sends its distal end or duct into this long papilla and opens alongside of the sac duct by a separate but closely approximated opening.

10. *Further experiments on the laterality of transplanted limbs.* Ross G. HARRISON, Osborn Zoological Laboratory, Yale University.

Additional experiments with the fore limb bud of *Amblystoma* embryos make it possible to state more simply than before the rules governing the laterality of transplanted limbs (Proc. Am. Assoc. Anat., Anatom. Rec., vol 10, 1916).

Limb buds were transplanted either to their natural location after removal of the normal bud (orthotopic transplantation), or to another region of the body, as for instance to the flank between the fore and hind limbs (heterotopic transplantation). They were grafted either on the same (homopleural) or on the opposite (heteropleural) side of the body, and were placed either in the upright (dorso-dorsal) or the inverted (dorso-ventral) position.

The following rules underlie the determination of the laterality of the appendages which develop out of the transplanted buds in both the orthotopic and heterotopic transplantations, though in the former

the limbs are more likely to be modified through the influence of their more normal surroundings (vascularization, innervation, etc.).

Rule 1. A bud that is not inverted (dorso-dorsal) retains its original laterality whether implanted on the same or on the opposite side of the body.

Rule 2. An inverted bud (dorso-ventral) has its laterality reversed whether implanted on the same or on the opposite side.

Rule 3. When double or twin limbs arise, as is frequently the case in these experiments, the original of the two limbs, i.e., the one first to begin its development, has its laterality fixed in accordance with the above rules, while the other is the mirror image of the first.

In heterotopic transplantations abortive development, or even complete absorption of the tissue, often takes place, and reduplication may occur in any of the combinations.

In the orthotopic series abortive development is more rare and reduplications, though frequent, are limited to certain combinations and may be further modified. The outcome in the several groups of experiments was as follows:

1. Homopleural dorso-dorsal grafts developed normally though at first very slightly retarded.

2. Homopleural dorso-ventral grafts resulted in:

- a. A single limb of reversed laterality (structurally and functionally perfect right limb on left side). One case only.

- b. Reduplicated limbs. More than half of the cases.

- c. Typical non reversed limbs which began their development by growing in abnormal direction, but ultimately assumed normal posture by rotation. These cases form the only exception to the rules and require further investigation.

3. Heteropleural dorso-dorsal transplantations yielded:

- a. Single non reversed limbs. Two cases only, neither perfect.

- b. Reduplicated limbs in which the secondary bud being reversed, has the laterality of its new surroundings.

- c. Cases similar to the above in their early development but differing later in that the reduplicating bud gained the upper hand and developed into a normal functioning limb of reversed laterality (corresponding to its new surroundings), while the original bud became reduced to a spur or appendage upon the other.

4. Heteropleural dorso-ventral transplantations developed into:

- a. Single limbs of reversed laterality somewhat retarded in their development (Rule 2). The great majority of cases.

- b. Duplicate limbs. A single case only.

Experiments with superimposed limb buds and with half buds, gave corresponding results, and together with other experiments, show that the mesoderm of the limb bud is an equipotential system, with definite asymmetry (laterality) which is subject to modification in accordance with the fundamental rules stated above. The theoretical questions involved, particularly those relating to adaptation in the individual, are of considerable interest.

11. *The effect of removal and regeneration of parts upon metamorphosis in amphibian larvae.* CHARLES ZELENY, University of Illinois.

The effect of removal and regeneration was studied by comparing the time of metamorphosis in a set of individuals subjected to operation with the time in a control set. Comparisons were made as follows:

Experiment 1. Rana. The effect on the early development of the hind legs of five successive removals and regenerations of the tail.

Experiment 2. Bufo. The effect of removal of the tail after the beginning of metamorphosis upon the time of completion of the process.

Experiment 3. Bufo. The effect of four successive removals and regenerations of the tail upon the time of metamorphosis.

Experiment 4. Bufo. The effect of two successive removals and regenerations of the tail upon the time of metamorphosis.

Experiment 5. Amblystoma. The effect of three successive removals and regenerations of the tail upon the development of the legs.

Experiment 6. Amblystoma. The effect of removal and regeneration of the tail upon the time of loss of the balancers.

Experiments 7 and 8. Amblystoma. The effect of removal of the right balancer upon the time of loss of the left balancer.

Experiments 9 and 10. Amblystoma. The comparative development of the legs in individuals subjected to the four following degrees of injury: (1) one fore-leg, (2) both fore-legs, (3) one-half of the tail, (4) one-half of the tail plus both fore-legs.

The data from these experiments give no indication that metamorphosis is delayed by removal and regeneration of parts of the body.

12. *Life history of Zeugophora scutellaris.* B. H. GRAVE, Knox College.

During the summer months the larvae work in the leaves beneath the epidermis, eating out the pulp and causing blackening of the parts affected. A large part of the chlorophyll bearing tissue may be destroyed in this way by the end of summer, thus rendering the leaf ineffective as a starch-making organ. The larva may therefore appropriately be called a leaf miner. Late in the season, at the time the leaves fall it crawls out and enters the ground. After burrowing to a depth of between $1\frac{1}{2}$ and $2\frac{1}{2}$ inches below the surface, it excavates a little spherical cavity in which it coils up for the winter sleep.

About the last of May of the following spring (May 25–June 15) the larvae transform into pupae. The duration of the pupa is about three weeks or possibly a month in cool weather. The first beetles appear by the middle of June. There is reason to believe that they appeared as early as June 10 in the year 1913, which was a rather early spring for that locality.

A number of beetles which were hatched from breeding boxes were kept in captivity and one of them laid eggs ten days after it emerged. It seems likely therefore that under normal conditions the eggs are laid upon the leaves and twigs during the latter part of June and the

first part of July and that the larvae enter the leaves soon after, and begin their destructive work.

The adult beetles, as might be expected, feed upon the leaves of the cottonwood. They swallow the softer parts and discard the fiber.

13. *Extirpation of the hypophysis and thyroid glands of Rana pipiens.*

BENNET M. ALLEN, University of Kansas.

Last spring a series of experiments was performed in the extirpation of the anlage of the anterior lobe of the hypophysis and of the anlage of the thyroid gland. The former experiment was successfully performed upon 430 tadpoles and the latter upon 336.

Removal of the anlage of the anterior lobe of the hypophysis produced a marked contraction of the black pigment cells of the integument apparent at the end of eight days. The silvery cells expanded giving the animals a bright uniform creamy silver color. At this time the hypophysis shows no evident histological differentiation. These operated tadpoles showed marked susceptibility to unfavorable conditions of the water. In the absence of the hypophysis the legs failed to develop, the hind legs appearing as mere buds, up to the maximum stage reached—tadpole of 30 mm. length.

Tadpoles deprived of the hypophysis were carefully studied in stages of 16.5, 21.5, 24 mm. comparisons being made with control tadpoles. The gonads, and thymus glands showed no consistent modification. The thyroid gland in all cases, however, showed a decided diminution in the amount of colloid produced. In tadpoles deprived of the hypophysis the colloid material occurred in far more irregular masses measuring from one-half to two-thirds the diameter of colloid masses in the thyroids of the controls. The general dimensions of the thyroid glands of these animals did not show any appreciable modifications at these stages.

The extirpation of the thyroid anlage caused the tadpoles to halt in their differentiation at a stage in which the hind-limb rudiments were but 4 mm. in length. At the end of November they had shown no further signs of differentiation. Two specimens from which the thyroid had been removed were fed thyroid extracts. In one instance with forty-five days thyroid feeding the hind legs grew to 9.5 mm. length and the fore-limbs grew to 5 mm. length as compared with 4 mm. and 2.5 mm. respectively in the thyroidless controls. They showed much greater differentiation of structure than found in those of the other thyroidless tadpoles. These also showed a remarkable shortening of the intestine in one case to 68 mm. as compared with 190 in the thyroidless tadpoles not fed with thyroid extracts. Studies are being made by students of mine upon the effects of thyroid removal upon the other glands of internal secretion and upon the skeletal system. Seven thyroidless tadpoles of gigantic size are still living, but show no signs of further differentiation.

14. *An experimental study of cell division.* L. V. HEILBRUNN, Department of Anatomy, University of Illinois, College of Medicine.

In the experimental study of a biological process, there are two general modes of procedure. One can attempt to artificially produce or initiate the process, or one can attempt to modify or block the process after it has started. In my study of artificial parthenogenesis, I adopted the former of these methods for studying cell-division. I attempted to analyze the effect produced by the various agents which cause the unsegmented egg to divide mitotically. More recently I have adopted the other method of attack, and I have studied the effect of various agents, which, without injuring the egg, prevent cell-division. Such a suppression of normal activity is of course an example of anesthetic action, and these experiments have incidentally furnished me with considerable data concerning the actual effect of various anesthetics upon the cell.

In a recent contribution it was shown that all agents which cause the egg of the sea-urchin to segment, produce a gelatinization in the cytoplasm. The details of this gelatinization process, as it occurs normally, have now been studied in the same egg. At frequent short intervals after fertilization, the viscosity of the egg cytoplasm was determined by the centrifuge method. After fertilization the cytoplasmic viscosity rises gradually until it reaches a maximum after about twenty to twenty-five minutes.¹ It is precisely at this time that the mitotic spindle first makes its appearance. The appearance of the spindle is followed by a gradual decrease in viscosity, the egg cytoplasm returns to its original fluid state. These viscosity differences are very marked and are easily measured. Similar series of viscosity changes during mitosis can also be demonstrated for the second cleavage. These facts in themselves lend support to the view that the spindle is coagulated out of the cytoplasm.

No doubt the gelatinization is confined to certain chemical constituents of the cytoplasm. On the other hand, it apparently extends throughout the cell and it is attached peripherally to the enclosing membrane, the so-called hyaline layer of the egg. Hence when the gelatinized egg is centrifuged, frequently parts of the egg surface are pulled in, and the shape of the egg commonly undergoes considerable distortion. This attachment to the enclosing membrane is retained by the astral rays of the spindle. Oftentimes when an egg which possesses a spindle is viewed from one pole, its surface contour does not appear perfectly smooth, but shows faint indentations at various points. These points probably represent the points of attachment of the astral rays. The inward pull exerted on the egg surface as a result of gelatinization, no doubt affords the best explanation of the decrease in cell volume which follows fertilization. This is borne out by the fact that in the *Cumingia* egg, decrease in volume does not immediately follow fertilization. In this egg both gelatinization and shrinkage only occur after a certain time interval has elapsed.

¹ Of course this time varies greatly with the temperature.

If the normal gelatinization of the cytoplasm is prevented, then the spindle never forms, the egg remains quiescent and does not divide. Even after gelatinization has begun, it may be reversed. Ether, chloroform, acetone, paraldehyde, propyl alcohol, isoamyl alcohol, ethyl butyrate, ethyl acetate, ethyl nitrate, acetonitrile, nitromethane, chloral hydrate, phenyl and ethyl urethanes, all prevent or reverse cytoplasmic gelatinization. In the study of the action of these anesthetics, the experimental procedure was usually as follows: soon after fertilization, the eggs were placed in a graded series of concentrations of the anesthetics. Then the viscosity of the various sets of eggs was determined. If the series of concentrations was well chosen, it was found that the highest concentrations produced coagulation, the lowest concentrations no effect, and the intermediate concentrations a liquefaction or reversal of gelatinization. As my studies progressed, I was soon able to predict the fate of the eggs. In the lower concentrations with no marked effect on the egg viscosity, the eggs would go on to divide, in the intermediate concentrations which produced liquefaction, development would be interrupted, but removal from the anesthetic (after a few hours), would be followed by a resumption of development. On the other hand, the coagulation produced by the higher concentrations was generally irreversible, although in a few cases the eggs thus coagulated were able to undergo a few cell-divisions on being returned to normal sea-water. The actual effect of the various anesthetics mentioned above, was to dissolve the lipoids of the egg, or at least to increase their degree of dispersion. In concentrations slightly above that best for anesthesia, the lipoids appeared to be completely dissolved, and no longer separated out when the egg was centrifuged. Such a solution of the lipoids was usually followed, after a short time interval, by coagulation of the cytoplasm.

Low temperatures (e.g., -5° to $5^{\circ}\text{C}.$) also prevent or reverse gelatinization. But this effect of cold is not due to the same cause as is the effect of the other anesthetics just mentioned. In fact when eggs are subjected to both cold and ether the effect of the one tends to counteract the effect of the other.

But not all anesthetics prevent gelatinization. Hypertonic solutions of various salts, although they act as anesthetics, produce quite the opposite effect on the cytoplasm. They intensify the normal gelatinization and in this way prevent cell-division. A similar effect is also produced by chloretone. The action of potassium cyanide is especially interesting. In concentrations of potassium cyanide, far above those sufficient to check development, the early stages of mitotic division are not suppressed. The spindle proceeds to form, but with its formation, development stops abruptly.² Apparently the cyanide renders irreversible the normal gelatinization process and no liquefaction follows. If eggs are placed into cyanide solutions during the

² The fact that the egg can begin its development in these solutions of potassium cyanide is a strong argument against the oxidation theory of artificial parthenogenesis.

anaphase stage of mitosis, after liquefaction has begun, then cell-division is not prevented.

Since the normal process of mitosis involves both gelatinization and liquefaction, it is easy to understand why agents which cause an intensification of either the one process or the other can check the division of the cell.

As to the direct cause of the gelatinization which follows fertilization, there is some evidence that it is due to salts rather than to an acid. Thus when the cytoplasm is diluted by endosmosis, the gelatinization is reversed. Moreover, whereas coagulation of the cytoplasm by hypertonic salt solutions can be reversed by ether, no other type of cytoplasmic coagulation can be so reversed. It is possible that the gelatinization is produced by the abstraction of water from the cytoplasm by the growing pronuclei. This gelatinization is then to a large extent reversed, when owing to the rupture of the nuclear membrane, the nucleus discharges its water back into the cytoplasm.

But these interpretations are more or less hypothetical. They do not stand on the same firm basis of fact as the observations: (1) That all agents which stimulate to cell-division produce gelatinization, and (2) that any agent which prevents or reverses this gelatinization, prevents spindle formation and cell-division. These two facts, taken together with the observed time relations of the gelatinization process, furnish strong evidence that the force which underlies spindle formation is a cytoplasmic gelatinization.

15. *Early castration of the vertebrate embryo.* FRANKLIN P. REAGAN, Princeton University (Introduced by C. F. W. McClure).

In 1870 Waldeyer advanced the view that the germ-cells of the vertebrate embryo arise in that portion of the coelomic mesothelium which covers the gonad. Since then a number of observers have described transitional stages between these epithelial cells and primitive ova.

Eigenmann ('91) is justly to be considered the leader of an opposing school who believe that the vertebrate germ-cells are of extra-regional origin. In the viviparous fish *Micrometrus*, he was able to trace the germ-cells probably to the fifth cleavage—certainly to a time prior to the closure of the blastopore, before there were distinct entodermal and ectodermal layers.

Hoffmann ('93) discovered primordial germ-cells in bird embryos which had not yet formed germinal epithelia. His work has been confirmed by a number of observers.

In connection with the work on avian embryos it is important to note that sex-cells could formerly be found only subsequent to the 22-somite stage; earlier than this their whereabouts was a complete mystery. In fact, stages transitional to them were here less evident than in pictures to be found later in the gonad. An interesting observation was that of Danchakoff, who found in the blood stream very large wandering cells of entodermal origin; these were believed to disappear at the age of 23 somites. Their disappearance was as mysterious as

the first appearance of the germ-cells. It required the insight of Swift ('14) to correlate these two facts and establish a morphological continuity of these large cells from the time of their proliferation by the entoderm, to their incorporation into the gonad. Swift found that these cells originate in a crescent-shaped area of entoderm anterior to the body axis of the very early chick embryo; that they enter the mesoderm which later invaded this region; that they enter the blood vessels forming here and are carried by the blood stream many of them to the base of the mesentery from which they migrate into the gonad.

In 1914, Prof. C. F. W. McClure suggested that the work of Swift could be proved or disproved by the early excision of this crescent-shaped area. I have been able to rear a number of embryos so treated to a stage in which it is possible to determine that the gonads are quite devoid of sex-cells. In normal individuals five days old, the mesenteric mesothelium adjacent to the gonad usually contains many large germ-cells which cause it to protrude locally. Also the mesenteric mesenchyme generally contains some of these big cells. The embryos treated as described have no trace of germ-cells in the gonads, and the neighboring mesothelium remains thin and barren of sex-cells. The stroma tissue of such gonads presents a peculiar foliage-like appearance; it is much vacuolated.

My own interest in this problem lies in another direction. It occurred to me that since castration of individuals subsequent to birth or hatching greatly affects the secondary sexual characters, embryonic castration might produce even more profound effects—that some sexual characters usually considered primary might really be secondary. Such a possibility is heightened by the recent work of Lillie on Freemartin. At present I am led to believe with Lillie that the fate of the Wolffian and Müllerian ducts is dependent on the internal secretion of the gonad. I believe further that if the stroma or interstitial cells are responsible for this secretion, they are unable to produce it in the absence of germ-cells in the early ontogeny; that the nature of this secretion is related only indirectly to the factor or factors for sex by way of emanations from the sex-cells themselves; that the only primary sexual character is the constitution of the germ-cells themselves. These points are not yet completely proved.

Attempts are being made to produce hermaphroditism by transplantation of these entodermal crescents. The effects thus produced on the ductless glands are also being investigated. So far, the transplantation of adult gonad-tissue to young embryos has had no effect on the embryonic genital system, though it has always caused hypertrophy of the spleen of the host.

I wish to thank Professors McClure and Conklin for confirming many of my observations on my material.

It was found impracticable here to discuss thoroughly the results of all previous observers.

16. *Microdissection studies. The cell aster: a reversible gelation phenomenon.* ROBERT CHAMBERS, JR., Cornell University Medical College. (Illustrated with drawing, to be used in projection apparatus.)

1. The centrosphere is an optically hyaline fluid area occupying the center of the aster and increasing steadily in size until the aster reaches full development. 2. The increase in amount of the centrospheric fluid is apparently due to the accumulation of fluid flowing into the centrosphere from all parts of the cytoplasm. 3. The aster rays are the channels along which the centripetal flow occurs. 4. The cytoplasm between the rays is in the gel state giving a certain amount of rigidity to the aster. The gel state is most pronounced centrally and peripherally passes gradually into the sol state beyond the confines of the aster. When the aster reaches the periphery of the cell the entire cell is comparatively rigid. 5. In the maturation figures of the egg nucleus the peripheral aster forms a continuous coagulum with the surface layer of the egg to which the entire figure is thus firmly attached. The confines of the central aster pass insensibly into the surrounding liquid cytoplasm. 6. A periodic reversal of the sol to the gel state and vice versa has been demonstrated in the cell protoplasm during division. The steps taken may be divided into the following series: *a.* When the monaster is fully formed the greater part of the cell is a gel. *b.* As the centrospheric fluid collects on the two poles of the nucleus the cytoplasm reverses to a sol state and the monastral radiations fade out. *c.* The formation of radiations about the centrospheres, one on each pole of the nucleus, produces the diaster and is accompanied by a return to the gel state. *d.* A return to the sol state later takes place in the equator of the cell. *e.* The nuclear spindle now divides followed by a constriction around the middle of the cell which continues until the cell is cut in two. 7. The reversal of the gel to the sol state usually starts in the equator of the cell and spreads to the poles. The reversal of the sol to the gel begin immediately about the centrosphere and spreads in all directions peripherad. 8. There are appreciable differences in the sol state of the cytoplasm in certain regions and at various times. The interior cytoplasm of the unfertilized and fertilized egg before the aster is formed is slightly viscous. The archoplasm in the centrosphere and in the rays also the hyaline area in the vicinity of the forming polar body are very fluid. 9. What is described as the gel state in living protoplasm cannot be considered as an inert solid coagulum. Even to the eye there is always a constant but very gradual change among the granules imbedded in the cytoplasmic gel.

One may conclude that one of the factors concerned in cell-division lies in the peculiar colloidal property of protoplasm, viz., a periodic reversibility in its sol and gel states.

17. *Multiple chromosomes of Hesperotettix and Mermiria.* C. E. MCCLUNG, University of Pennsylvania.

A restudy of the chromosome complexes of *Hesperotettix* and *Mermiria*, upon greatly enlarged collections, has made possible the correction of some errors in the earlier account and the discovery of important new facts. Upon the basis of the present understanding of conditions in the germ cells of the Orthoptera, numerical variations of the chromosomes are found to be a strong support to the individuality hypothesis instead of militating against it.

The multiple chromosome of *Mermiria bivittata*, at first thought to be a decad, because of certain constrictions and even divisions in metaphase, proves to be a hexad like the one in *Hesperotettix*, consisting of a tetrad joined to the accessory chromosome. An explanation of its form and behavior became possible upon the discovery of the J-shaped tetrads in *Trimerotropis* by Carothers. Full collections make the determination of numbers in the different cell generations certain and consistent with the interpretation of the multiple as a hexad. So far, conditions within the species appear to be constant, but others than *bivittata* may not have multiple chromosomes, e.g., *neomexicana* and *texana*. Within the taxonomic group *bivittata*, as at present constituted, there are certain sub-groups, first distinguished apart by the form of the multiple chromosomes, that appear to be specifically distinct upon careful study of somatic characters.

As in *Mermiria*, a study of extensive series of specimens of *Hesperotettix* shows that the universality of multiples in all species, a condition realized in my earlier collections, does not exist. Moreover the constancy of occurrence within the species sometimes is lacking, as in *viridis*, and this is associated with a tendency to form multiples between certain of the euchromosomes, producing octads, a name given to such structures in an earlier paper ('05) in advance of their realization in experience. The presence or absence of multiples in *viridis* results in variations of numbers from 9 to 12 in the first spermatocyte and a supernumerary in one individual out of 37 studied raised the upper limit to 13. The number 11 may be constituted in three different ways. Despite the apparent lack of definiteness in organization suggested by the range in numbers, there is abundant evidence that fundamental conditions are not thus disturbed, because if the final mitotic units, the chromatids, are considered the same 46 are present in each first spermatocyte except in the one individual with a supernumerary. All the evidence indicates that the constitution of the individual established on zygotis are maintained, for no variation was found within the individual.

The chromosome conditions of the first spermatocytes so far observed fall within six different classes; (1) Twelve separate chromosomes consisting of eleven tetrads plus the accessory chromosome dyad—a total of forty-six chromatids; (2) eleven separate chromosomes, ten tetrads plus a hexad—again forty-six chromatids; (3) ten separate chromosomes or eight tetrads plus one hexad plus one octad—

forty-six chromatids; (4) nine separate chromosomes or six tetrads plus one hexad plus two octads—forty-six chromatids; (5) again ten separate chromosomes but in this case consisting of seven tetrads, plus two octads plus one accessory chromosome dyad—forty-six chromatids; (6) again eleven separate chromosomes but consisting of nine tetrads, one octad and the accessory chromosome dyad, forty-six chromatids. In the individual with a supernumerary there are eleven separate chromosomes with the other condition similar to class 5 above.

Criteria for resolving the varying numbers of free metaphase chromosomes into identical series of units of lower order are furnished by comparisons of form, size and behavior and by the structural conditions of the elements. The conditions in class 1 are typical for large numbers of the short horned grasshoppers, the earliest modification of which appeared in the first members of the genus *Hesperotettix* which I studied, representing class 2. Here the easily recognizable accessory chromosome is joined to a first spermatocyte tetrad, producing a hexad which acts as a unit in this mitosis. Because of this mitotic relation the hexad is properly called a chromosome, but it is just as definitely different from the other members of the complex, for the morphologically distinct accessory chromosome exhibits all its structural peculiarities quite as clearly as if it were a free unit, while the associated tetrad passes through its usual history. The principle of chromosome union is thus definitely and unequivocally established. Only in very recent material have the conditions in classes 3, 4, 5 and 6 been encountered. These involve an extension of the principle of chromosome association to combinations between two tetrads in the first spermatocyte and a persistent union of the non-homologous elements involved throughout the cells of the individual. These combinations result from the endwise union of contiguous sized members of the complex. Thus in class 3 the largest two tetrads are so united, while in class 4 the next two in size also form into another octad. In every case the joined tetrads clearly exceed in size the next in order, just as they do in the free condition, and as the gradation in the complex requires. In synopsis and during the first spermatocyte prophase their behavior is not altered by the association. Union may involve one or both ends of the tetrads producing rings or V's. In the latter case first spermatocyte anaphase groups differ accordingly. Such conditions are accounted for upon the assumption of persistent chromosome individuality and chance union of the classes of gametes actually seen to form. Similar combinations between the accessory chromosome and a tetrad in the long horned grasshoppers were reported by me ('02) and more recently between euchromosomes ('15) by Robertson and by Woolsey. The conditions are therefore not abnormal but represent the action of definite forces of chromatin integration. No evidence is at hand to show how such associations arise or to indicate their later dissolution. The facts demonstrate that numbers are reduced by definite and gradual steps of which the chromosomes are the measure. The normal number is not exceeded by the addition of other

normally constituted chromosomes. Supernumerary chromosomes when present, show their aberrant nature by extra-nuclear position, irregular behavior and great variability. Persistent association of non-homologous chromosomes with elimination of intermediate stages would produce a permanent reduction in the number of free chromosomes. It is possible to account for the occurrence of the lesser number in *Stenobothrus* in this way, but the criteria for this determination, urged by Robertson, are not completely valid since V-shaped chromosomes with achromatic bridges occur in complexes where no reduction in number exists.

18. *The spermatogenesis of Culex pipiens L.* P. W. WHITING, University of Pennsylvania, (Section F).

In the spermatogonia of *Culex pipiens* there are three pairs of V-shaped chromosomes, the members of which are usually approximated. Before division the pairs always lie parallel. One of the pairs is smaller than the other two.

In the first spermatocytes three characteristic tetrads appear, any one of which may form either a cross or a ring. The four elements of the tetrads are distinguishable in late prophase and in metaphase. The dyads separate into monads in the anaphase.

Nucleolar elements are found in spermatogonia, in first and second spermatocytes and in spermatids. In spermatogonia they are associated with one of the large pairs; in first spermatocytes, with a large tetrad; and in second spermatocytes, with a large dyad.

19. *The segregation and recombination of homologous chromosomes in two genera of Acrididae (Orthoptera).* E. ELEANOR CAROTHERS, University of Pennsylvania.

The subjects of this report are the chromosome conditions in the male germ cells of certain species of two closely related genera of short-horned grasshoppers.

A microscopical study of these cells has shed light on three points: (1) the manner of segregation of morphologically distinct homologues, (2) the zygotic composition of the species in regard to these dissimilar homologues and (3) a possible cytological basis for separating confused species of the two genera.

1. The chromosomes are constant in number, size and shape in each animal. In size there is the usual double series, one homologue of each pair being derived from each parent. Contrary, however, to anything heretofore reported these homologous chromosomes may differ in shape—one being a straight rod, the other V-shaped. In one species seven of the twelve first spermatocyte chromosomes may be composed of such dissimilar homologues. This peculiarity affords an opportunity to trace the segregation in the gametes of certain chromosomes derived from both parents. It was found that this segregation occurred according to the law of chance.

2. A study of the chromosome complexes of ninety-five individuals, both male and female, showed the zygotic composition of the species to

be such as would result from the random union of these gametes. These facts furnish an extensive physical mechanism for the operation of Mendel's laws of heredity.

3. One of these genera, *Circotettix*, has eleven chromosomes in the haploid series of all species studied instead of the usual twelve as found in the other genus, *Trimerotropis*; on this basis the debated species, *Circotettix suffusus* which has twelve chromosomes, should be changed to *Trimerotropis suffusa*.

20. *Synapsis and chromosome organization in the male germ cells of Chortippus and Trimerotropis.* D. H. WENRICH.

In a recent paper¹ the writer showed that pairing of chromosomes in the first spermatocytes of *Phrynotettix magnus* is by parasynapsis. All the chromosomes of *Phrynotettix* are of the rod-shaped type. A study has been made of the first spermatocyte chromosomes of *Chortippus* (*Stenobothrus*) *curtigennis* which has three pairs of C-shaped and five pairs of rod-shaped chromosomes, and of *Trimerotropis siffusa*, which Dr. E. Eleanor Carothers has found to possess not only pairs of rod-shaped and pairs of V-shaped chromosomes but also pairs consisting of one rod-shaped and one V-shaped chromosome. In all cases the mode of synapsis is found to be the same, viz., a side-to-side union. This mode of synapsis makes it impossible to determine whether the first or the second maturation mitosis is the segregating division unless there is a recognizable difference between the conjugants of a pair.

The V-shaped chromosomes of both *Chortippus* and *Trimerotropis* may have the point of spindle-fiber attachment indicated by a constriction, or by a small non-chromatic region, or by both. Since *Trimerotropis* has a larger total number of chromosomes and (usually) a large number of V-shaped chromosomes than *Chortippus*, the existence of this "weak place" cannot safely be taken as an indication that these chromosomes are compound.

In *Chortippus* the point of fiber attachment of the V-shaped chromosomes may be further marked by the presence of a small, appendant, plasmosome-like body, which, when present, is constant in this position on the chromosome. Other such bodies are found on two of the rod-shaped chromosomes of *Trimerotropis*. Their positions are constant for each chromosome. The constancy of position which these bodies exhibit with reference to the chromosome to which they are attached is additional evidence of the constancy of organization possessed by the chromosomes.

21. *The chromosome complex in Apithes agitator.* W. J. BAUMGARTNER, University of Kansas.

Apithes agitator is a small brown cricket often called the shrub cricket. It lives on various shrubs, especially the coral berry.

¹ Wenrich, D. H. 1916. The spermatogenesis of *Phrynotettix magnus* with special reference to synapsis and the individuality of the chromosomes. *Bull. Mus. Comp. Zool.*, Harvard College, Vol. 60, pp. 57-133, 10 plates.

The chromosome complex is very instructive as the number is small and the individual chromosomes are quite distinct in size and shape and behavior.

In the spermatogonia the number is thirteen, two small straight rods, and eleven U-shaped rods. The largest of these always lies on the periphery of the group and its end may be swollen or split or bent at a sharp angle. This has no mate and is the accessory. The other ten easily group themselves into five pairs. The largest pair show a tendency to lie in or toward the center of the equatorial plate. They are frequently somewhat straightened, sometimes showing a double-wave-like curve. The other eight chromosomes with the accessory usually lie in a circle forming the periphery of the equatorial plate. All have the free ends extending outward in the typical way. There is an evident tendency for the two of a pair to lie near together. In size these pairs grade down from nearly as large as the largest to about half of its size.

In the spermatocyte the accessory appears as a sausage-shaped rod, and behaves as described in my earlier papers and confirmed by other observers. The small pair are now a longer rod. It frequently shows a constriction and divides precociously. The two dyads may have separated even before the larger tetrads have been drawn into the equatorial plate of the spindle. The other ten chromosomes now form five very definite rings. All of these enter the spindle parallel with the direction of fibers and not in the plane of the plate as most of the rings do in the grasshoppers.

The fiber attachment is terminal in the small chromosomes, and median or nearly so in all the others. In dividing one end sometimes separates before the other so that the tetrad may appear like a printed capital C for a short time. Sometimes the sides of the rings (ends of dyads) are swollen. When such a tetrad is seen from the side it may appear as a cross with a very short cross arm. But in either the cross or C shapes the fundamental shape is a ring.

The seven elements in Apithes are one accessory sausage shape, one small rod and five rings lying in the plane of the spindles fiber. I believe these shapes are constant, i.e., are assumed in every cycle of tetrad formation. I think this fact is a strong evidence of chromosome individuality.

The five large rings may be multiple chromosomes. If each is counted as a double multiple then the original number would be 5 times 4 plus 2 rods plus 1 accessory equals 23. This number is found in some crickets and most grasshoppers, and seems to be kind of a basic number for these families. Several other species of crickets would have this number if these large rings were counted as double multiple chromosomes. But in two species studied this number is not obtained if the rings are so counted. The interpretation of the large rings as multiples can be stated only as a probability.

22. *New facts and views concerning the occurrence of a sexual process in the life cycle of a myxosporidian Chloromyxum leydigii.* RHODA ERDMANN, Osborn Laboratory, Yale University.

In a myxosporidian life cycle the sexual process is generally believed to be *before* spore formation. After a shorter or longer asexual life in which the formation of a vegetative body with either two or many nuclei is effected spore formation begins. Since 1910 Auerbach and Erdmann ('11) have verified the suggestion of Doflein that the sexual process might not occur at the above mentioned place in the life cycle but as soon as the young animal leaves the spore. Auerbach and Erdmann found young animals (amoeboideum) which had left the spore and possess either two or one nuclei. They are the first step in the new life cycle. Erdmann ('11) could further produce these young forms in a culture which had been made on *gall plates*. In my recent work, finished in 1913, which does not appear until 1916 in consequence of the war, I figure those young animals experimentally freed from the spore after fixation and staining.

Besides myself two other authors, Georgevitch ('14) and Davis ('15) assure that at the beginning of spore formation *no sexual process* could be found. It shows that the recent investigators have finally left the old view that the sexual process of myxosporidia occur immediately *before* spore formation. To support this view I can point out that all processes which were thought to be sexual, i.e., the formation of residual (reduction) nuclei and the heteropole division at the first beginning of spore formation, are only connected with the development of the spore membrane. The *glykogen* which I could point out to be present in the vegetative myxosporidian body, is used up during spore formation. The small cells and their nuclei being the product of the above mentioned heteropole division form the membrane of the spore. Also smaller or bigger chromatic lumps (residual nuclei) are extruded by the nuclei of the sporoblast-cells and are used in forming the sporogenous membrane, and the polar bodies. They cannot be thought to be *reduction* nuclei.

Having pointed out what I believe to be the real significance of these processes, it is more in accordance with the facts presented that the sexual process in the life cycle of myxosporidia is to be found in the beginning of the life cycle after the young animal left the spore.

23. *Spermatogenesis in the albino rat.* EZRA ALLEN, University of Pennsylvania (Section F).

The haploid, or reduced number of the chromosomes is fundamentally nineteen. The spermatogonial number is consistently 37. There is one accessory. This divides in the second spermatocyte division. One chromatoid body in the cytoplasm and one nuclear plasmosome are present. There is no evidence of double reduction. The chromosomes are of different sizes. In the spermatogonia the forms assumed are rods; in the first spermatocytes, rods, crosses, rings, and single and double loops; in the second spermatocytes, they are all rods. The

organization and behavior resemble the orthopteran. The first spermatozoa are ripe when the rat has reached the age of forty days. The most satisfactory method of fixing and preparing the material for study is described in a paper giving the results of his experiments on technique published by the author in the *Anatomical Record*, vol. 10, p. 565.

24. *Multiple complexes in the alimentary tract of Culex pipiens.* CAROLINE M. HOLT, University of Pennsylvania (Section F.)

1. During metamorphosis in *Culex pipiens*, the number of chromosomes in the cells of the larval intestine is considerably increased.

2. Before disintegration of the cells begins, the chromosomes of each larval gut cell pass through a number of longitudinal divisions resulting in three or four multiplications.

3. The number of chromosomes in the multiple complexes is always a multiple of three—oftenest 6, 12, 24, 48; but frequently 9, 18, 36, and even 72 may appear.

4. The triplex divisions of the chromosomes apparently arise through premature splitting of one member of each pair of daughter chromosomes from the original complex of three bivalents or by a precocious division of one of each of the homologous elements of bivalent chromosomes.

5. The size relation between nucleus and cytoplasm is extremely variable.

6. It appears that in the resting stage of those cells of normal size which contain multiple complexes, there must be an accelerated growth of each chromatin thread which splits into normal sized chromosomes in prophase, or else the cytoplasm of such cells must fail to divide and to grow while the chromosomes continue to do both. The former seems to be the probable explanation.

7. There is evidence of a parasynaptic union of sister chromosomes in the resting stage, followed by reseparation through longitudinal splitting in the prophase.

8. These sister chromosomes, the multiples of each member of the original complex, tend to remain together throughout the mitotic changes.

9. The individuality of the chromosomes is maintained until the cell disintegrates.

10. The disintegrated cells appear to be digested by the cells of the newly formed lining of the adult alimentary tract.

All these facts suggest that increased metabolism of the older epithelial cells may be a means of supplying needed food material to the developing cells of the adult gut during the pupal changes. That this great increase in the amount of chromatin in cells which have attained their growth, functioned for a time, and are about to be absorbed, is not accidental, or simply a process of degeneration seems reasonably clear from the uniformity and universality of this increase in the intestine of *Culex*. Every cell of the larval gut epithelium apparently passes through the whole series of changes above described

before it reaches the stage of disintegration. If this were simply a process of degeneration, it would be hard to account for the tremendous growth in the chromatin material and for the retention of the individuality of the chromosomes to the end. One would expect the processes observed in the disintegration of the cells, to come directly without these elaborate preparatory phenomena. It would seem that we have here not the hit-or-miss phenomena of degenerating cells, but a definite adaptation to provide for the support of the organism during metamorphosis.

25. *Sex-linked inheritance of spangling in poultry.* GEORGE LEFEVRE, University of Missouri.

Spangling is the term applied by poultry fanciers to the occurrence of a well-defined spot or "spangle" of distinctive color at the tip of the feather. In the breed of Silver Spangled Hamburgs, for example, the feathers are white and each is tipped with a black spangle which is generally proportionate to the shape and size of the feather. The color-pattern is the same in both sexes.

A series of experiments has been carried out for the purpose of determining the mode of inheritance of spangling, as it was thought that so definite and simple a color-pattern would be favorable for genetic analysis. It is the experience of breeders, however, that the spangled pattern is not reproduced closely.

The initial crosses were made reciprocally between Silver Spangled Hamburgs and Brown Leghorns, and the material used for the analysis has been obtained from twelve different matings.

The conclusion has been reached that spangling is determined in inheritance by a distinct factor which behaves in a typically sex-linked fashion, the cocks being homozygous and the hens heterozygous, for it in Silver Spangled Hamburgs. When spangling is introduced through the male, both sexes in the F_1 generation show spangles, while the reciprocal cross gives only spangled males, the females being non-spangled and incapable of transmitting the pattern.

It has been further shown that the expression of spangling may be greatly modified, or even entirely obscured, by the action of other factors, especially factors for black pigmentation, which, however, segregate independently of the factor for spangling.

The disturbing factors may affect the entire body or only some restricted region, as, for example, the feathers of the tail. Black pigment, moreover, may be present in the feathers in other parts than at the tip, and in varying degrees may obscure the definiteness of the spangles. In fact, in certain individuals the condition is reached in which black is developed to such an extent as to completely cover the body, even in cases in which the bird carries the spangling factor, as may be proven by appropriate breeding tests.

In the light of the above facts, it would seem probable that multiple factors for black, introduced by the Brown Leghorns, are present

and that these factors may have a cumulative effect, with the result that pigmentation is developed to varying degrees of extension.

The independence of the spangling factor is shown by the fact that, after segregation and recombination of the several factors concerned, some individuals are extracted in which all disturbing factors are absent and the spangled pattern is exhibited in its original purity. A number of such birds have been obtained from different matings, and these now breed as true to spangling as do the Silver Spangled Hamburgs themselves.

26. *Two classes of factors for color patterns in Paratettix.* ROBERT K. NABOURS, Kansas Agricultural College.

Fourteen factors for patterns, each allelomorphous to the other, have been used in Paratettix breeding experiments. In this class two factors for any one suffice to make the whole pattern, and two for different ones produce a hybrid pattern, intermediate in fact, though the one may be more apparent (epistatic) and the other less apparent (hypostatic). These are well established as multiple allelomorphs. *Each of these factors is invariably allelomorphous to a multiple allelomorph, never to an absence.*

Another class of factors, existing without exception in connection with and in addition to the multiple allelomorphs, has been discovered. The one factor most studied produces a well marked melanism in addition to any of the other patterns or their hybrids. It is also possible to distinguish in the patterns between the presence of a single and double dose of the factor. *This factor is allelomorphous only to its absence and never to anything.*

The multiple allelomorphs among themselves produce the typical 1 : 2 : 1 (in some cases apparently 1 : 3) ratios. When the non allelomorphous factor is present the typical 9 : 3 : 3 : 3 : 1 (actually 1 : 1 : 1 : 1 : 2 : 2 : 2 : 2 : 4) ratios are secured. The clear definition of the patterns and the ratios indicate the presence of only one pair of allelomorphous (allelomorphous each to the other) factors, and one unpaired (allelomorphous only to its absence) factor in the production of the 9 : 3 : 3 : 3 : 1 ratios. This conception applies perfectly to similar phenomena in other forms. Considering one example in peas: it appears that the factors for roundness and wrinkledness are each allelomorphous to the other, while the factor for yellow is unpaired and allelomorphous only to its absence and never to anything. It is completely misleading to assume that the factor for yellow forms an allelomorphous pair with green. Another case is that of combs of fowls: pea and single appear to form an allelomorphous pair, and there is another factor, behaving as the unpaired one for the melanism in Paratettix and yellowness in peas, which, when present, modifies single to make rose (the one rose being single heterozygous for this factor, and the other kind of rose being single homozygous for it), and which modifies pea and the hybrid of pea and single to make the four kinds of walnut. It is misleading to consider rose a character; it is a modified single.

Pea with heterozygous and homozygous doses, respectively, of the modifying factor makes two kinds of walnut, while the hybrid (pea and single) with heterozygous and homozygous doses, respectively, of the modifier makes the other two kinds of walnut.

The data and fuller discussion are in press.

27. *The relation of yellow coat color to black-eyed white spotting of mice, in heredity.* C. C. LITTLE, Harvard Medical School.

It has been known for some time that mice homozygous for the factor producing yellow coat color have never been observed. Castle and Little ('10) showed that the ratio of yellow to non-yellow young when two yellow mice are crossed together is approximately 2:1. This is explicable on the ground that the homozygous yellow individuals are formed but fail to develop.

A similar condition has been found to exist in the case of the factor producing black-eyed white spotting in mice. When two black-eyed whites are crossed together they produce approximately two black-eyed whites to one ordinary piebald mouse. Black-eyed whites are always heterozygous and carry the ordinary type of spotting as an hypostatic character.

A series of experiments have been carried on to determine whether or not the lethal action of the yellow factor and the black-eyed white factors is identical. It has been shown that they are entirely distinct in nature. This is proved by the breeding tests of the classes of young produced, by the size of litters, and by the ratio of yellow to non-yellow animals in F1. In the course of the experiments it became evident that in both "black-eyed white" and piebald animals which are "yellows" the amount of dorsal pigmentation is from 5 to 30 per cent greater than "non-yellows" of the same two color types. As yet, there is no evidence as to whether this is due to interaction of the yellow and black-eyed white factors or whether it is due to some distinct genetic factor linked with yellow color.

28. *Mutation in Didinium nasutum.* S. O. MAST, Johns Hopkins University.

In a series of experiments on the effect of conjugation and encystment in *Didinium* extending from April, 1910, to May, 1914, there suddenly appeared a marked difference in the rate of fission in the progeny of a single individual. This difference appeared in the latter part of July, 1912, in a line which had at that time produced 721 generations without conjugation and 197 generations without encystment. The difference was still evident, apparently without diminution, when the experiment was closed after having continued 315 days. There was great variation in the rate of fission from day to day depending largely upon changes in temperature, but the difference in the rate of fission in two groups of lines remained fairly constant throughout.

During the 315 days over which the experiment extended the more rapid lines produced a total average of 838 generations and the less

rapid lines a total average of 634 generations. The death-rate in the two groups was nearly the same, as was also the tendency to encyst and to conjugate.

29. *The occurrence of mutations in skunks of the species, Mephitis putida and M. hudsonica.* J. A. DETLEFSEN. Coll. of Agr., Univ. of Ill.

Eleven mutant skunks of the species *Mephitis putida* and three mutants of the species *M. hudsonica* have been found. In the former species we have, or had, in our possession four living individuals and received hair samples of two other mutants. In the latter species we have one living mutant and hair-samples of two other mutants. The mutants are as follows:

Mephitis putida

Female: White hair on body; few brown hairs on face; eyes black. Successfully bred to normal male, producing three normal offspring.

Female: White hair; eyes pink except a narrow ring of pigment on the outer margin of the iris. Successfully bred to normal male, producing seven normal offspring.

Two female albinos with pink eyes (in our possession).

Male albino, an albino of unknown sex and two solid blacks of unknown sex have been reported to us. Male and two female brown skunks (hair samples received.)

Mephitis hudsonica

Albino male: White hair and pink eyes (in our possession).

Male and female brown skunks (hair samples received).

30. *The influence of parental alcoholism on the learning capacity of the offspring.* E. C. MACDOWELL, Carnegie Institution of Washington.

Rats from alcoholized parents have been compared with their double first cousins from normal parents. No structural differences between the rats of normal and alcoholic parentage have been found. The number of rats in a litter averages slightly lower in the matings of alcoholics. Three methods have been employed in rating the capacity for learning:

1. Puzzle box. To enter, the rat must release the door by going behind the box and breaking an electric current; results based primarily on the time of operation.

2. Yerkes multiple-choice apparatus. The problem is to choose the correct door, from a variable series of opened doors, according to its relationship to the other opened doors; results based on the numbers of correct first choices, and the numbers of wrong choices.

3. Watson circular maze. Five choices of going to the right or left are offered; the correct path is followed on choosing right and left turns alternately, results based on time, and camera lucida tracings of the distances run in every trial.

Three groups of litters have been studied. These include 64 rats from normal parents, 54 from alcoholic parents. All the rats in each group are double first cousins.

Group 1. Tested with the puzzle box, the rats of alcoholic parentage, are faster; tested with the multiple-choice apparatus, two litters in this group show the normals to be the better choosers.

Group 2. Tested with the puzzle box the rats of alcoholic extraction are faster.

Group 3. Tested with the puzzle box, the normal rats are faster than those of alcoholic parentage; tested with the multiple-choice apparatus the rats from normal parents are again more successful; two litters in this group tested with the maze, show that the rats from alcoholic parents are faster and cover less ground in learning.

51. Linkage in the sex-chromosome of a new species of Drosophila. (Introduced by C. B. Davenport.) CHAS. W. METZ, Carnegie Institution of Washington.

In an undescribed species of *Drosophila* several sex-linked mutants (as well as several non-sex-linked ones) have been obtained, and have been studied for linkage. The factors for these sex-linked characters fall into a linear series when arranged according to their linkage relations, in much the same manner as factors have been shown to do in *Drosophila ampelophila*. By means of this series it is possible to make a comparison between corresponding linkage groups, and perhaps even between corresponding individual factors, in two related species.

52. An examination of the so-called process of contamination of genes. THOMAS HUNT MORGAN, Columbia University.

A sex-linked mutant factor in *Drosophila* called Notch produces two effects, a notch in the wing (dominant) and a lethal effect (recessive) so that no notch males ever appear. In the heterozygous females the notch varies between a well-marked serration at the end of the wings to an occasional fly with wings having the normal margin. Through several generations females were selected that had the least amount of notching and then through several generations more females were selected that had notch in only one wing. Such females had both the normal factor and the notch factor, but the notch character was so slightly developed somatically that it showed only on one wing. To those who believe that a somatic character can be used as a measure of the 'potency' of the factor affecting the character, this one-sided development would appear to fulfill the conditions nearest to 'genic weakness.' Finally, the character was carried further in stock that had one other recognizable factor close to Notch on each side, so that the character could be followed by its linkage even after it had been selected into invisibility, i.e., normal winged flies that carried the factor could be selected and the stock bred from them. Suitable tests will be pointed out by means of which one can find out

whether selection has accomplished its result by piling up modifying factors, or by the isolation or allelomorphic mutations, or perchance by causing 'allelomorphic fluctuations' occasioned by the 'contamination' of the genes.

33. *An analysis of the effect of selection on bristle number in a mutant race of Drosophila.* A. H. STURTEVANT, Columbia University.

A mutant race of *Drosophila*, known as 'Dichaete,' was found to be variable in the number of bristles present on the thorax. A selection experiment has been carried out on this character, involving over 25,000 flies and extending through fourteen generations. Both plus and minus races have been obtained. These races have been tested, by means of the linkage method, to see if the differences between them were due to modifying factors or to changes in the *Dichaete* gene itself.

34. *The elimination of males in alternate generations of sex-controlled lines.* CALVIN W. BRIDGES, Columbia University. (Introduced by T. H. Morgan.)

There have been demonstrated in *Drosophila* sex-linked genes, which kill all males receiving such genes. Recently a sex-linked lethal (lethal 10) has been found which allows an occasional male with the lethal gene to come through as a pale-colored dwarf. These rare dwarfs are fertile and transmit to all their daughters the lethal gene and consequently the power to produce only half as many sons as daughters. By mating a lethal 10 dwarf to a female carrying another lethal (lethal 12) whose locus in the X chromosome is exceedingly close to the locus of lethal 10, females are obtained that are incapable of producing any sons except the rare dwarfs, although producing 200 or 300 daughters.

35. *Coincidence of crossing over and the chromosome theory of linkage.* ALEXANDER WEINSTEIN, Columbia University. (Introduced by T. H. Morgan.)

It has been found in *Drosophila* that a crossing over in one region of a chromosome tends to prevent crossing over in a neighboring region. This has been termed interference. The likelihood that one crossing over will interfere with another decreases with increase of distance between them. The present evidence indicates that if this distance becomes sufficiently great, interference disappears entirely; and as the distance increases still further, interference reappears. These results have a definite bearing on the twisting of the chromosomes. They are in accord with the chromosome theory of linkage, and any other theory must be able to explain them.

36. *Determinate and indeterminate laying cycles in birds.* L. J. COLE, University of Wisconsin.

There appear to be two distinct types of laying cycles in birds, one in which the number of eggs which will be laid in the clutch is definitely

determined when laying begins and the other in which the number of eggs that will be laid depends upon stimuli received after laying has begun. In other words the stimulus for cessation of laying and inseption of brooding has already been received and the reaction predetermined in the first case, while in the second the stimulus is received later and is followed by cessation of liberation of ova from the ovary, though laying continues for a time afterward until the ova already discharged have received albumen and shells and have been expelled. The most important stimulus for the onset of broodiness, and the consequent cessation of laying, in the second class of cases is probably a physiological reaction of the female to a number of eggs in the nest. As a consequence, if the eggs are removed as laid the stimulus does not occur and laying continues beyond the regular clutch to an indefinite number.

Among domesticated birds the pigeon may be taken as an example of the determinate type and the common fowl of the indeterminate. Among wild birds experiments have been carried on with the English sparrow and the house wren, which also appear to represent the two types respectively.

37. *A strain of sex intergrades.* ARTHUR M. BANTA, Carnegie Institution of Washington.

From an individual brought into the laboratory in August, 1912, several separate strains of *Simocephalus vetulus* have been propagated for more than 150 generations. Only parthenogenetic reproduction can have occurred for—in the first place individuals are isolated when released from the mother's brood pouch—long before the sexual products are matured; in the second place for 130 generations no males or fertilizable eggs appeared in this stock; and in the third place the individual culture bottles are not retained long enough for a fertilized egg to develop if fertilized eggs were produced. Hence there are three reasons, any one of which is sufficient in itself, for stating positively that sexual reproduction cannot have occurred in this strain for 130 generations. There can, then, be no question of a recent hybridization within this stock.

In addition to the character of the gonads (primary sex characters) which is readily determined by examination of the living animal with the microscope, eight definite morphological secondary sex characters are recognized.

In October, 1915, in one of the six strains of this line there suddenly appeared sex forms in a remarkable array and this array of peculiar sex forms has persisted for more than 25 generations. None of the other five strains coming from the original mother more than 150 generations, and more than four years ago, has to date produced males. The strain producing the sex forms is not inferior to its sister strains in vigor or productivity.

The sex array in the sex intergrade strain consists of normal females, female intergrades having one to eight male secondary sex characters,

hermaphroditic intergrades with various combinations of male and female primary and secondary sex characters, male intergrades with one or more female secondary sex characters, and normal males.

Roughly the various sex forms fall into the classes indicated but really no precise and definite classification is feasible. Almost every possible combination of primary and secondary male and female secondary sex characters occurs. There is nearly every gradation between a normal female and a normal male. A single individual, even a single gonad, may produce eggs and sperm at the same time or sperm at one time and eggs at another.

There is a distinct, though not very precise, relation between the secondary and the primary sex characters. Usually an individual with most of its secondary sex characters male will have testes, and conversely an individual with most of its characters female will have ovaries. However many female intergrades have five or six male characters. Some have all their secondary sex characters those of a male.

Male intergrades usually produce few sperm or have incompletely developed reproductive systems. Female intergrades with all the secondary characters male are sterile. Those with as many as six male characters as usually sterile, while those with four or five male secondary characters are frequently sterile or show a much reduced productivity. Sterile individuals begin to develop eggs but they either disintegrate in the ovary or die in the brood pouch. Females with few male characters are usually normal in vigor and productivity. The proportion of the various sex forms produced by the different mothers varies greatly but in general those mothers which are themselves intergrades produce a larger percentage of males and intergrades than normal females in this strain.

Sex here is obviously a purely relative thing. There exists a graded series from normal females, to female intergrades with one to several of their secondary characters those of a male, to hermaphroditic intergrades with various combinations of primary and secondary male and female characters, to male intergrades with, in some cases several, in other cases a single female secondary sex character, to normal males. Maleness and femaleness are not definite and fixed mutually exclusive states but are quantitative and purely relative things.

38. *Effect on fertility of crossing closely and distantly related stocks of Drosophila ampelophila.* ROSCOE R. HYDE, Indiana State Normal School.

The fertility of *Drosophila ampelophila* is probably very high in nature as shown by the fact that about twenty wild stocks taken from widely separated regions have given a fertility of from 80 to 100 per cent when tested in the laboratory. These stocks when inbred have shown without exception a decline in fertility. The per cent of eggs that give rise to mature flies has dropped in some stocks to as low as 25. When these stocks are crossed a marked rise in fertility above that of the parent stocks used for control occurs both in the cross and its reciprocal.

This result stands out in marked contrast to that obtained when a stock is divided into strains and separately inbred. In this case the strains may loose different degrees of fertility. When the stocks are recombined there is no rise in fertility beyond the parent stock with the highest fertility. The control strain with the highest fertility brings the fertility of the lower strain up to its level but not beyond.

59. *Are the polyradiate cestodes mutations?* FRANKLIN D. BARKER, University of Nebraska.

It is not surprising to find terata or "freaks" commonly among such erratic and degenerate animals as the cestodes or tapeworms. Of these abnormal forms the most rare and in many ways the most unique are the polyradiate or "double" cestodes having the appearance of two worms variously fused giving rise to two, three or more "wings" or sides. These are known as dihedral, trihedral and polyhedral cestodes. Such terata have been found among the cestodes of man, horse, dog and cat.

One of the most interesting aspects of these abnormalities, as with all terata, is their origin. A number of theories have been advanced by various parasitologists to account for their origination such as, the partial fusion of two normal adult cestodes (Bremser); the primitive malformation of the scolex with subsequent partial fusion of two worms (Vaillant); the fusion of two normal embryos which give rise to a double adult (Leuckart); several helminthologists have described these anomalies as distinct varieties of the normal species while others have even gone so far as to consider them as new and distinct species (Kuckenmeister +).

A similar anomaly has been reported among six species of cysticerci or larval cestodes and an abnormal number, more than six hooks, is frequently found in the onchosphere or first larval stage of practically all species of cestodes.

Foster ('15) has recently reported the results of experimentally feeding to a rabbit, two gravid segments of a triradiate *Taenia pisiformis* "shipped in a solution of formaline of unknown strength, and kept in a 2 per cent solution of formalin for one week." Seven cysticerci were found fully grown and entirely normal.

We published in Science, 1910, the finding of specimens of trihedral *Taenia serrata* = (*T. pisiformis*) and *Taenia serialis*. Unfortunately the specimens had been killed and fixed before we discovered their trihedral character.

On July 29 of this year we had the good fortune to find a perfect mature trihedral *Taenia serrata* in the intestine of a collie dog picked up on the streets of Lincoln, Nebraska. The last two gravid proglottids of the living worm were teased in physiological salt solution and 3 cc. of this solution containing the freed eggs was fed to each of three half-grown rabbits born and raised in our laboratory pens. Three rabbits from the same litter were kept as checks. August 2 one infected rabbit died from unknown cause. No trace of cysticerci was found.

August 13, one of the uninfected rabbits died from unknown cause. No cysticerci were found. October 16 one of the infected rabbits was killed and 31 cysticerci were found attached to the omentum, liver and posterior end of the colon. Twenty-six of the cysticerci had well developed scolices and 5 were immature. Sixteen cysticerci were removed from their protective cysts and placed in 200 cc. of digestive fluid made up of distilled water, 0.2 per cent pancreatin, 0.6 per cent NaCl, and 5 per cent saturated solution sodium carbonate and kept at blood temperature, 37°C. in an incubator for ninety minutes. The 11 mature cysticerci completely evaginated their scolices in from thirty to ninety minutes. All scolices were perfectly normal with respect to number of suckers and hooks. October 17 16 cysts were fed to a 6 months' old dog. November 18, dog was killed and 3 hookworms (*Uncinaria trigonocephala*) and 1 *Taenia serrata* were found firmly attached to the wall of the lower end of the small intestine. The cestode was perfectly normal though immature, measuring in a relaxed condition 5.5 cm. The last two proglottids were asymmetrical indicating their original nature. The small number of worms found indicates a lack of vitality of the cysticerci, while the absence of other parasites commonly found even in puppies points to a natural immunity or an unfavorable condition of the intestine. November 27 the third infected rabbit was killed. Six cysts were found, 4 attached to the omentum and 2 to the colon. Five cysticerci were mature, and all were perfectly normal. November 28 the two remaining uninfected rabbits were killed but no cysts were found. The results of these feeding experiments prove conclusively that the eggs of polyradiate cestodes develop into normal worms and do not give rise to polyradiate forms and therefore are not mutants, distinct varieties or species, but are terata or abnormalities which probably arise, as we have previously suggested, from the occasional partial and incomplete separation of early blastomeres of embryos of normal cestodes.

40. *A revised working-hypothesis of mimicry.* W. H. LONGLEY, Goucher College.

There is grave reason to doubt the existence of animals whose conspicuousness under normal conditions has been exaggerated by natural selection. It seems desirable, therefore, to attempt to discover what explanation of mimetic resemblance is possible upon the assumption that the colors of insects are correlated with their habits, tend to reproduce characteristic tones of their surroundings, and to obliterate their possessors under the conditions in which they live.

It is to be noted that this supposition is perfectly consistent with the many verifiable facts that have been discovered by special students of mimicry. Upon the basis it provides, it is to be anticipated that mimic and model should commonly be found living under the same conditions; that certain groups of insects should show the same local color varieties; that diversity of coloration should appear in one group of butterflies or moths and ally them in outward appearance with dif-

ferent genera; and that insects with every variety of larval experience, as adults should possess the same type of coloration, and superficial resemblance which they attain in the most diverse fashion. The same may apparently be stated of all other admissible evidence, which has been assumed to prove the validity of the Batesian and Müllerian hypotheses.

Upon the other hand recognizable deficiencies in current explanations of mimicry, and criticisms levelled against them, seem adequately met by the revised hypothesis. There is no longer any difficulty in comprehending how even the initial stages in mimetic resemblance could minister to the advantage of their possessor. One's credulity is no longer overtaxed by the demand to believe that when the patterns of scores of species of one locality present a single combination of colors, they reveal the effect of natural selection directed toward the production of resemblance. One is able to escape the inquisitor who wishes to know how creatures, which were capable of being deceived by the first vague resemblance between two species, have been able byselection to push the agreement between the two to the point of apparent identity, and even the occurrence of the 'mimic' beyond the range of its 'model' is capable of rational explanation.

It is therefore suggested, as a working-hypothesis, that mimicry has been initiated and advanced by indiscriminate feeders. These have exerted bionomic pressure, and forced their accustomed prey to assume color combinations which most effectually conceal it in its normal environment. In the evolution of types of coloration appropriate to the surroundings and habits of their possessors members of one genus have occasionally followed different courses, and fortuitous resemblances to various unrelated genera have occurred, capable of deceiving enemies, which exercise discrimination in their choice of food. At this point selection directed to the production of deceptive resemblance has been superimposed upon processes culminating in the development of types of obliterative coloration without changing the general trend of their evolution.

41. *Recent studies of nerve conduction in Cassiopea.* ALFRED G. MAYER.

Researches conducted at The Tortugas Laboratory of the Carnegie Institution of Washington upon *Cassiopea* indicative that nerve conduction in this medusa is due to a chemical reaction involving the cations of sodium, calcium, and potassium; magnesium being relatively non essential.

The sodium and calcium cations together appear to combine with some undetermined proteid element to form an ion-proteid. The probably high temperature coefficient of ionization of this ion-proteid may account in some measure for the high temperature coefficient of the rate of nerve conduction, which is 2.5 as great as that of the electrical conductivity of the sea water surrounding the nerve.

The rate of nerve conduction is probably accelerated by an enzyme as stated by E. N. Harvey, 1911.

R. S. Lillie, 1916, *American Journal Physiol.*, vol. 41, p. 133, appears to be mistaken in assuming that the rate of nerve conduction is a function of the electrical conductivity of the solution surrounding the nerve, for the decline in rate of nerve conduction is practically identical whether we dilute sea water with 0.415 molecular $MgCl_2$ or with distilled water. In other words whether we maintain a constant electrical conductivity or reduce it in a ratio nearly commensurate with the dilution. Thus Lillie's "local action" theory appears not to be supported.

My former idea that adsorption of Na^+ , Ca^{++} , and K^+ played an important rôle in nerve conduction is erroneous. I was misled by the effects due to a slight acidity of the distilled water, and I did not reduce all observations to a constant temperature, which is essential, due to the high temperature coefficient of the reaction.

42. *The theory of sex as stated in terms of results of studies on the pigeons.*

OSCAR RIDDLE, Carnegie Institution.

Studies which have demonstrated the reality of the control or reversal of sex in pigeons (Whitman, and later Riddle) have at the same time indicated the *nature* of the initial difference between germs of prospectively different sex-value. This difference rests upon different *levels of metabolism*; and when the metabolic level of a given germ is shifted from the level characteristic of the germ of one sex, sufficiently toward the level of the other sex, it develops into an organism of the sex which corresponds to the acquired, or later, level.

The *initial* difference characteristic of the two kinds of (sex) germs, tends to persist and characterize the adults of the two sexes.

Sex is based on a quantitative difference; intermediates of the normal extremes have been experimentally produced, and the normal extremes have themselves been experimentally accentuated.

There seems to be no known body of facts in contradiction of this view; though the facts obtained from the pigeons are in direct contradiction of some of the more or less current theories.

43. *The adaptive color changes of tropical fishes.* W. H. LONGLEY, Goucher College. (Illustrated with lantern.)

The colors of tropical reef-fishes are correlated with their habits. Their color changes may occur almost instantaneously, and in many species even those of unconfined individuals are subject to direct control by the observer. In general, they enable the animals that display them to repeat upon their own bodies the characteristic tones of the various environments in which they move.

Photographs secured with a submarine camera and diving-hood record some of the changes in coloration which *Epinephelus striatus* and *Lachnolaimus maximus* commonly exhibit, and indicate their effect in reducing the creatures' conspicuousness. Others show how particular elements in a complex pattern such as that of *Abudefduf saxatilis* blot out their possessor's contour, when the animal is viewed against an appropriate background.

A great body of evidence indicates that more than minimal conspicuousness may not be ascribed rationally to bright colored fishes as a class, and strikes at the foundation of some of the most widely disseminated hypotheses of animal coloration. Obviously, however, it suggests that the characters in question are useful, and that their development has been largely controlled by natural selection.

44. *The histological basis of adaptive shades and colors in the flounder, Paralichthys albiguttus.* ALBERT KUNTZ, St. Louis University School of Medicine.

Changes in shade and color in fishes are due primarily to changes in the distribution of the pigment granules in the chromatophores in the superficial layers of the skin and changes in the relationships of the guanophores (cells containing guanin crystals) with these chromatic organs.

The skin of *Paralichthys albiguttus* contains chromatophores of two distinct types, viz., melanophores and xanthophores. The former contain melanin granules which vary in color from dark brown to black, the latter contain xanthine granules which vary in color from yellow to orange.

Under experimental conditions pigment granules can be observed advancing toward the periphery and in turn retreating toward the center of the chromatophores along more or less definite radial lines. Ameboid movements of the chromatophores can not be observed in adult specimens. Neither could evidence be obtained from preparations of the skin which indicates that the chromatophores contract and expand in an ameboid manner. Changes in the distribution of the pigment in the chromatophores are accomplished by movements of the pigment granules within the cells and do not involve essential changes in the form of these organs.

A comparative study of living material and preparations of the skin of specimens of *Paralichthys albiguttus* adapted to backgrounds of various shades indicates that shade depends primarily upon the degree of distribution of the melanin pigment in the melanophores and the spacial relationships of the guanophores with these bodies in the superficial layers of the skin. The xanthophores probably play no important part in the determination of shade.

The most obvious response to a change in the color of the background is a change in the distribution of the xanthine granules in the xanthophores. Shades of yellow and orange depend primarily upon the degree of distribution of the xanthine granules in the xanthophores containing yellow and orange pigment respectively. In general the particular quality of the color assumed by the fish depends upon a complex group of factors which do not lend themselves readily to a detailed analysis. Some of the colors assumed may be duplicate by mixing pigments of the colors represented in the pigments contained in the chromatophores. These colors depend primarily upon the degree of distribution of the pigment granules in the melanophores

and xanthophores respectively. Colors which can not be duplicated in this manner, doubtless, depend upon the relative degree of distribution of the pigment granules in the melanophores and xanthophores plus the optical effects due to the diffraction of light by the guanin crystals in the guanophores. The optical effects produced by the guanin crystals are probably modified by the particular spacial relationships of the guanophores with the chromatic organs.

Obviously, certain colors are simulated by the fish more perfectly than others. Among the colors used in the present investigation yellow and green were simulated more perfectly than dark red and dark blue. None of the specimens placed on a dark red background showed any color which approximated the color of the background more closely than the orange pigment in the xanthophores. In view of these facts the conclusion that all colors can be reproduced in the skin of the flounder is unwarranted.

Adaptation to a yellow background involves a moderate degree of concentration of the pigment in the melanophores and a marked degree of distribution of the pigment in the xanthophores in the superficial layers of the skin.

Adaptation to a green background involves a marked degree of distribution of the pigment in the melanophores and a moderate degree of distribution of the pigment in the xanthophores in the superficial layers of the skin. The resultant yellowish green color probably depends upon the ratio of the distribution of the xanthine to the distribution of the melanin pigment plus the optical effects due to the diffraction of light by the guanin crystals in the guanophores.

Adaptation to a dark red background involves almost maximum distribution of the pigment in the melanophores and the orange colored xanthophores and a marked degree of concentration of the pigment in the yellow xanthophores in the superficial layers of the skin. The resultant reddish brown color is due largely to the wide distribution of orange pigment, the effect of which is probably modified by the blending of orange and black and the optical effects produced by the guanophores.

Adaptation to a dark blue background involves almost maximum distribution of the pigment in the melanophores and almost maximum concentration of the pigment in the xanthophores in the superficial layers of the skin. Many of the guanophores also become arranged with reference to the melanophores and closely associated with them. Doubtless, the resultant greenish blue color depends largely upon the optical effects produced by the guanophores which are closely associated with the melanophores. The dark shade is due to the wide distribution of the melanin pigment.

45. *Further data on the relation between the gonads and the soma of some domestic birds.* H. D. GOODALE, Massachusetts Agricultural Experiment Station.

The results of published data on the ablation of the testes and ovary of domestic birds together with unpublished data on the transplanta-

tion of the ovary into castrated males has made it clear that different parts of the soma react in different ways to the secretion of the gonads. Each character appears to be more or less independent of every other character just as they are more or less independent in heredity. The various characters fall into several groups. We may recognize, first, characters (including some of the secondary sexual characters) that are independent of either ovary or testis. Such characters are, size in the female, voice and some phases of behavior, and mandible color in ducks. Second, characters affected by the testis, such as comb and wattles, fat deposition, size in the male, and some instincts and summer plumage in ducks. Third, characters that are affected by the ovary, such as plumage form and color and some phases of behavior.

No sharp line can be drawn between the better known secondary sexual characters and those commonly considered ordinary somatic characters, for the reaction of a character to the secretion of either gonad varies not only according to the character itself but also according to the original sex of the individual. Thus, the size of the primary coverts in relation to the primaries is approximately the same in each sex but after removal of the testes they become disproportionately large, though not after removal of the ovary. The spurs always develop in the female after removal of the ovary, but they also develop in the capon and in feminized cockerels, i.e., in the presence of the ovary in the soma of the male. Females from which the ovary has been removed are neutral in sexual behavior but one of the most astonishing things about castrated males with implanted ovaries is that they exhibit only the sexual behavior of the male. The comb and wattles do not develop in the male after castration, i.e., are infantile; in the castrated male with engrafted ovary they are fully feminized; in the ovariectomized female they may be either female-like or male-like. The capon exhibits two characters that are female-like, viz., the amount of fat deposited and the brooding instinct. On the other hand, two masculine characters are intensified by the removal of the testes, viz., body size and plumage length. The ovariectomized female is approximately the size, however, as her normal sisters, while the castrated male with engrafted ovary has the size of the male. The plumage shape seems to be completely controlled by the ovary, since wherever that is present the shape of the feather is like those found on intact females. The color is less completely controlled by the ovary for while females from which the ovary has been removed develop the colors of the male, only a portion of those males with engrafted ovaries have developed the female's color, though they do not develop all the colors of the male, particularly the brilliant colors. In ducks there is a greenish pigment of the mandible that disappears from the mandible of the castrated female, but since the mandible color of the male with engrafted ovaries is unaltered, it appears that castration induces a previously non-existent difference between the sexes. Finally, there are characters that behave one way in some individuals and in another way in others. This is particularly noticeable in the plumage of the ovariectomized ducks, which in particular

regions may vary from a purely masculine condition to a purely female condition or to a condition *sui generis*.

If the entire series of altered individuals is examined, it is apparent that it may be looked upon as a series of sex intergrades. That is, characters that are normally found in one sex may be experimentally transferred to the opposite sex while individuals composed of mixtures of such characters may be obtained.

46. *The sensory potentialities of the nudibranch 'rhinophore.'*¹ LESLIE B. AREY, Northwestern University Medical School.

Nudibranch mollusca possess a pair of short, robust dorsal tentacles which are commonly perfoliate or ringed and which may or may not be retractile. These important looking tentacles have long been designated 'rhinophores,' and it is tacitly assumed that they are indeed specialized olfactory organs. The presence in certain species of long, more or less dorsally placed tentacles, in addition to the oral tentacles and rhinophores, heightens the suspicion that the latter may perhaps serve some particular sensory function.

The sole experimental evidence upon which the assignment to the rhinophores of an olfactory activity rests seems to be found in the observations of Graber in 1877 (Biol. Centralbl., Bd. 8, No. 24, pp. 743-754). Graber brought oil of rose near the head of *Chromodoris elegans* and observed the withdrawal of the rhinophores to be quicker and more vigorous than that of the oral tentacles. He emphatically states, however, that the post-branchial region is the most sensitive part of the body.

It would thus appear that the convenient term 'rhinophore' is of dubious propriety. For this reason Bermudian nudibranchs were subjected to experimentation designed to test their sensory potentialities. Unless otherwise stated the following account applies to *Chromodoris zebra* Heilprin.

Tactile stimulation. When a rhinophore is touched lightly with a glass rod it is jerked back precipitately within its protecting collar. The sensitivity of the rhinophore to gentle stimulation is astonishing and the explosive type of response is, within wide limits, independent of the strength of the stimulus. Fatigue comes on but slowly, responses of somewhat diminished intensity being readily obtained after fifty successive stimulations at ten-second intervals.

The oral tentacles, gill plumes and the general body-surface all respond to tactile stimulation. It is unsatisfactory to list the several regions of the body in the order of their sensitivity, for the types of responses are not all comparable. It appears, however, that the so-called rhinophore is the most sensitive part of the body and considerably more so than the oral tentacles.

Thermal stimulation. The head region and especially the oral tentacles react distinctly to water at 40°-50°C. applied with a pipet.

¹ Contributions from the Bermuda Biological Station for Research, No. 52.

The rhinophores, on the contrary, give faint and rather doubtful responses except to temperatures as high as 50°C.

Chemical stimulation. Equal volumes of various chemical solutions were applied from a constant distance with a pipet. Solutions of 1 M of maltose, or sucrose, or M/2 of lactose were without effect upon all parts of the body, although 3 M glycerine did evoke general responses. Several alkaloids had very weak effects or none at all. Alcohols and organic acids in concentrations of M/10 called forth strong general responses. The chlorides of the alkali metals Na, K, NH₄ and Li likewise stimulated the body in general, the rhinophores and oral tentacles, however, showing the greatest sensitivity. Solutions of substances which produce in man the taste sensations recognized as acid, bitter, salty and alkaline were applied in various concentrations. The gills fail usually before other parts, although all responses gradually weaken with increasing dilution.

From the foregoing tests it becomes evident that the rhinophore is not only extremely sensitive to chemical stimulation of diverse sorts, but that this sensitivity is only second to, if indeed it does not equal, that of the oral tentacles, which from their position might be suspected *a priori* of a specialized gustatory or common chemical function.

Olfactory stimulation. Saturated solutions in sea water of numerous essential oils and decoctions of decaying marine invertebrates were prepared and applied by the pipet method. The rhinophores react strongly to these solutions, but other parts of the body appear to be, so far as one can judge from the dissimilarity of the responses, equally sensitive. When a drop of oil is held for some time midway between the rhinophores no response ensues. If the rhinophore, or body, be touched gently with a drop of pure oil, the reaction is weaker than to a saturated solution. Here the number of sense organs stimulated is undoubtedly a complicating factor, yet there is suggested further that the response is one to an olfactory, rather than to an irritative or 'smarting' stimulus.

An essentially similar behavior to odorous substances was found in *Chromadoris roseapicta*, *Elysia crispa* and *Fiona marina*. Besides the rhinophores, *Facelina goslingi* possesses long, more or less dorsal tentacles, and short oral ones. The longer pair reacts more vigorously to solutions of the oils than do the rhinophores.

Summary. The entire body of *Chromodoris zebra* is sensitive to mechanical, common chemical, gustatory and olfactory stimuli. The head region is somewhat responsive to the application of increased temperature. Several other nudibranchs exhibit general olfactory sensitivity. Of the various parts of the body, the rhinophore is most sensitive to touch; is second, if not equal to the oral tentacle, in its response to chemical stimulation; and shares its sensitivity to odorous substances with the oral tentacle. In at least one species, *Facelina*, the long posterior tentacles react more vigorously to solutions of essential oils than do the rhinophores. Only to thermal stimulation is the rhinophore (of *Chromodoris*) clearly inferior in sensitivity. Hence

the so-called rhinophore, like the insect antenna, is a compound sense organ, for which the misleading term 'rhinophore' is highly inapt.

47. *Paramacium* grown in pure cultures of bacteria. GEORGE O. HARGITT and WALTER W. FRAY, Syracuse University.

Identical hay-infusions, inoculated with bacteria of the air, hay, or tap-water, produced flora quite similar for air and hay inoculation, somewhat different for water inoculation. The bacteria present in the infusions after a growth of three weeks were somewhat different than at first; at the end of three months entirely different types of bacteria had developed and some of the original forms had disappeared. In general it was found that the bacteria present in the fresh cultures were more favorable as food, and those of a three months culture were generally unfavorable as food for *Paramecium*.

From fresh and old cultures, from normal and abnormal (fermenting and putrefying) cultures, over thirty different kinds of bacteria were isolated in pure cultures; of these eleven were identified and their morphological, cultural, and bio-chemical characteristics were thoroughly studied. Sister *Paramecia* were grown in pure cultures of these eleven kinds of bacteria and their favorableness or unfavorableness as food was determined by the rate of division of the protozoa. No single kind of bacteria was as satisfactory a food as a mixture of different kinds of bacteria. Some bacteria were so unsatisfactory as to cause the death of *Paramecium* more quickly than if the protozoa were grown in sterile water. *Bacillus subtilis* was the best single form for food; in some cases *Paramecium* feeding on them divided more rapidly, in other cases less rapidly than on a mixed diet.

By using bacteriological methods it was demonstrated that *Paramecium* could be rendered absolutely sterile by washing the animals through five or six changes of sterile fluid. All feeding tests were carried on with sterilized *Paramecia* of the same strain. All pipettes, slides, moist chambers, and the like were sterilized in a hot air sterilizer at a temperature of 150°C., or more. The culture medium used was made at one time, placed in test tubes and sterilized before the experiments were started; the fluids were therefore identical throughout the investigation. All external conditions were controlled, or at least were the same for all cultures, so that the only difference between the experimental cultures was in the food supplied to *Paramecium*.

The feeding experiments were conducted as follows: A sterile depression slide was filled with hay infusion in which were growing bacteria of a single sort (a pure culture of bacteria). A single *Paramecium* was introduced and the slide placed in a sterile petri-dish, the atmosphere of which was kept moist by a small amount of sterile water. Each day some of the originally prepared, standard, hay-infusion, freshly inoculated with the desired bacteria, was placed in a slide in a sterile moist chamber and one *Paramecium* from the slide culture of the previous day transferred to it with a sterile pipette. The slide culture for a feeding experiment was usually carried for a period of ten days or two weeks.

If proper precautions were taken the culture fluid of the slide at the end of the period of experimentation was generally found to contain only the single kind of bacteria originally introduced. This was determined in each case by making an agar plate of the culture fluid at the end of the experiment. Occasionally strange bacteria gained entrance and contaminated the culture fluid, but the contamination was so slight that the diet of *Paramecium* was probably not modified to any extent. In the most extreme case a count of the bacteria at the end of the experiment (two weeks) in the slide culture showed the contaminating bacteria present in the ratio of 1 to 350 of the form originally introduced.

The results of the feeding experiments are believed, therefore, to be accurate indications of the effect of different kinds of bacteria as food for *Paramecium*.

48. *Recognition among insects.* N. E. McINDOO, Bureau of Entomology, Washington, D. C.

It has always been a matter of conjecture as to how the various lower animals recognize one another and by what means the sexes of any species distinguish each other. The senses of sight and touch are undoubtedly used considerably for this purpose, but it is probably true that the olfactory sense is the most important factor. Jaeger (*Zeitsch. f. wiss. Zool.*, Bd. 27, 1876, p. 322) goes so far as to assert that most animals emit odors peculiar not only to the individual, variety, race, and species, but also to the genus, family, order and class, and that these odors are the chief means by which one animal recognizes other animals.

The experimental results embodied in the present paper deal only with the odors emitted by honey bees. Some of the results are not conclusive, although the following are now fairly well established by von Buttel-Reepen and the present writer.

It is certain that a queen gives off an odor, and it seems reasonable that the odors from any two queens would be slightly different. All the offspring of the same queen seems to inherit a particular odor from her. This odor, called the family odor, perhaps plays little or no use in the lives of bees, for it is certainly masked by the other odors. Drones seem to emit an odor peculiar to their sex, but little can be said about it. It seems certain that each worker emits an individual odor which is different from that of any other worker. It is also probable that the wax generators and nurse bees emit odors slightly different from those of the field bees.

Of all the odors produced by bees, the hive odor is probably the most important. It seems to be the fundamental factor or principle upon which the social life of a colony of bees depends, and perhaps upon which the social habit was acquired; without it a colony of bees could not exist. The hive odor is composed chiefly of the individual odors from all the workers in a hive, and is supplemented by the odors from the queen, drones, combs, frames and walls of the hive, etc. From

this definition it is easily understood why no two colonies have the same hive odor. The hive odor of a queenless colony is perhaps considerably different from that of a colony which has a queen. The absence of a queen odor in the hive odor probably explains why the workers in a queenless colony are irritable and never work normally. All the bees—workers, queen and drones—in a colony carry the hive odor of that colony on their bodies among the hairs. This odor serves as a sign or mark by which all the occupants of a hive “know” one another. Since the queen and drones are “aristocrats,” they seem to disregard the sign that has been thrust upon them, but whenever a queen enters the wrong hive, she soon “realizes” that she wears the wrong badge. Worker bees returning to the hives from the fields pass the guards unmolested, because they carry the proper sign, although the hive odor that they carry is fainter than when they left the hive, and it is also partially masked by the odors from the nectar and pollen carried by these bees.

Bees kept in the open air for three days lose all the hive odor carried on their bodies, but each bee still emits its individual odor. When a colony is divided the hive odor in each half soon changes so that by the end of the third day the original colony possesses a hive odor so different from that of the other half of the colony, that when the workers are removed from the two new colonies and are placed together in observation cases, they fight one another as though they had been separated all their lives.

While a foreign hive odor calls forth the fighting spirit in workers, the queen odor always seems pleasant to workers regardless of whether the queen belongs to their hive or to another hive. Even though the queen odor forms a part of the hive odor, it is probable that this odor to the workers stands out quite prominently from the hive odor. That workers do not miss their queen for some time after she has left the hive, indicates that her odor thoroughly permeates the hive odor and that whenever this odor grows faint the workers “know” that she is not among them.

There has been much speculation concerning the ruling spirit or power in a colony of bees. The writer is inclined to believe that a normal hive odor serves such a purpose. The hive odor is a means of preserving the social life of the bees from without, the queen odor which is a part of it insures continuation of the social life within. As already stated the workers “know” their hive-mates by the hive odor they carry. This odor insures harmony and a united defense when an enemy attacks the colony. The queen odor constantly informs the workers that their queen is present, and even though she does not rule, her presence means everything to the bees in perpetuating the colony. Thus by obeying the stimuli of the hive odor and queen odor, and being guided by instinct, a colony of bees perhaps could not want a better ruler.

49. *The rate of locomotion of vanessa antiopa in different luminous intensities and its bearing on the "continuous action theory" of orientation.*

WM. L. DOLLEY, Jr., Randolph-Macon College.

If orientation in light is dependent upon the stimulation of both retinas by equal amounts of light, energy as is held by Loeb and his "continuous action theory," butterflies should move more rapidly in bright light than in weak. To test this the rate of movement of 10 specimens of *Vanessa antiopa* in each of two lights, one about 2,000 times stronger than the other, was ascertained. They did not move faster in the bright light than in the weak, but, on the contrary, 70 per cent of the insects actually moved more rapidly in the weak light than they did in the strong. These results support those presented previously which indicated that the orientation of *Vanessa* in light cannot be accounted for on the basis of Loeb's theory. Moreover, some positive evidence has been obtained in favor of the theory that orientation is dependent upon the time rate of change of intensity, since the results of some experiments seem to indicate that *Vanessa* moves faster in intermittent light than in continuous light.

50. *A super-organ for the expansion of Renilla.* G. H. PARKER, Harvard University.

The Pennatulid *Renilla*, in its adult state, consists of a flat, kidney-shaped portion, representing the expanded rachis, and a peduncle of striking proportions. The dorsal surface of the rachis is said to carry three kinds of zooids: large autozooids capable of considerable expansion and generally scattered over the surface; small siphonozooids also generally scattered; and a single axial zooid situated on the axis of the colony and not far from its middle. The peduncle has long been known to be divided longitudinally into two chambers by a delicate membrane.

If a specimen of *Renilla* is roughly handled, all its zooids contract and its volume is much diminished by the loss of seawater. Reëxpansion is accomplished by the peristaltic action of the walls of the peduncle whereby the colony becomes refilled with seawater. The seawater enters through the mouth of the axial zooid; it then passes down one chamber in the peduncle to the neighborhood of the distal end of that part where it crosses through apertures in the membrane to the opposite chamber. From this chamber it makes its way by appropriate channels to the various autozooids which are thus expanded. The pressure under which this water flows is due to the muscular contractions of the peduncle. The peduncle in *Renilla*, and probably in many other Pennatulids, is therefore an organ of expansion for the whole colony.

If the functional parts of protozoans are to be called organoids, and the functional parts of metazoan individuals organs, the functional parts of metazoan colonies, such as the peduncle of *Renilla*, may be called super-organs.

51. *The photoreceptors of amphioxus.*¹ W. J. CROZIER, Bermuda Biological Station.

An incandescent filament appropriately mounted was substituted for the ocular on one limb of a binocular microscope; by means of this device it was possible to secure, with properly adjusted diaphragms, an intense beam of light, microscopic in diameter, which was focussed within or upon various portions of *Branchiostoma carribaeum*. The exact location of the light-spot, and the extent of light scattering by the tissues, were observed through the other tube of the binocular. Practically every portion of the body of a number of lancelets was examined in this way in a dark-room; precautions were taken to avoid mechanical stimulation, to which *amphioxus* is very sensitive in the dark. No responses were obtained except when the light was focussed upon or within the ventral half of the nerve cord. It was possible to prove, notably by experiments with individuals in which portions of the integument were thoroughly anaesthetised, that this stimulation did not concern photoreceptors in the skin.

The integument of *amphioxus* therefore contains no normal photoreceptors. As indicated by Parker's less precise tests, the optic cups within the nerve-tube are probably the light-sensitive organs in this animal. This conclusion is substantiated by the details of the illumination trials, and particularly by the demonstration of photo-mechanical changes in the pigment cups of the "Sehzellen." Some evidence was secured which points to the photosensitivity of the "dorsal Sehzellen" of Joseph. The region of the anterior pigment spot is insensitive to light.

52. *The olfactory reactions of snails.* MANTON COPELAND, Bowdoin College.

It is well known that certain snails have the habit of collecting about decaying organisms or living bivalve mollusks, in the latter case often causing considerable damage to oyster beds. The reactions to food of two species belonging to the genera *Alectrion* and *Busycon* (*Sycotypus*) were carefully studied with the view of determining the general nature of the response, the manner in which food is located and the sense organ concerned.

Alectrion which showed variation in its rheotropic reactions moved more often against the current when a dead fish was placed at the head of the stream, and extended its proboscis in search of food when stimulated with fish juice squirted from a pipette. *Busycon* exhibited similar reactions to an extract of oyster. Since the response was obtained from dilute juices emanating from distant food, it may be regarded as truly olfactory.

The tentacles of gasteropods have been described as olfactory organs, but both species studied showed the same type of reaction after their removal. The snails had the habit of burying themselves in the sand, leaving only the tips of their siphons exposed. Under such conditions they often came out of the sand when food juices were taken

¹ Contribution from the Biological Station for Research, No. 53.

into the siphons. It soon became evident, therefore, that the olfactory organ was situated either within the siphon or mantle chamber. Since Alectrion still reaction to juices after the greater portion of the siphon had been removed, it seemed probable that the osphradium was the olfactory receptor. This conclusion was substantiated by scraping away the osphradia from several specimens of *Busycon*, which then failed to show olfactory response, although they took food when it was tasted and in other ways exhibited normal behavior.

The way in which *Busycon* locates distant food through its olfactory sense was definitely determined. When the snail is moving the siphon is continually swinging from one side to the other. If the stimulus is applied when the siphon is at the end of its movement to the left the foot turns in the same direction, whereas it turns to the right if stimulation occurs at the termination of the dextral swing. There is no difficulty, therefore, in leading a snail in any direction over the floor of the aquarium or up its side by applying oyster juice to the tip of the siphon, provided the organ is first pointed in the direction which it is desired the animal shall follow. When two cheesecloth bags, one containing a piece of oyster, were fastened in front of and lateral to the siphon tip, one on the right the other on the left, the snail turned in the direction of the baited bag, in a single instance completing two and a half circles.

The foregoing experiments show that the snail instead of possessing the paired olfactory organs characteristic of most animals has a median one situated near the base of the siphon. Accordingly, orientation to dilute chemical stimuli involves two distinct muscular activities, first a right and left swinging of the siphon preceding stimulation, and secondly a movement of the foot in the direction indicated by the siphon at the time it conducts the stimulating materials to the sense organ, the osphradium. By this procedure the snail continues to move toward and finally arrives at the source of the stimulus, its food.

53. *The reactions of the crimson-spotted newt, diemyctylus viridescens, to light.* A. M. REESE, University of West Virginia.

1. Phototropic reactions of *Diemyctylus* are markedly negative; in 30 observations 251 were found in the dark to 95 in the light half of the aquarium.

2. At temperatures near freezing water the animals become so sluggish as to be more or less indifferent to light; if the temperature be raised to about 36°C. they become abnormally active, and are again indifferent to light; at 40°C. they are seriously affected or even killed.

3. These animals respond in the same way, though less markedly, when half of the aquarium is illumined from below.

4. *Diemyctylus* is positively phototactic towards even very weak daylight, such as is seen on a cloudy day 20 feet away from an ordinary window.

5. With a 25W 115V Tungsten light 6 inches from the end of the aquarium 298 animals faced the light to 90 that faced away from it;

and 244 were noted in the near half of the aquarium to 163 in the far half.

6. With an electric arc projection lantern 15 inches from the end of the aquarium 116 animals faced the light to 41 facing away; and 105 animals were in the near half to 60 in the distant half.

7. At low temperatures the phototactic response to white light is inhibited or even reversed; i.e., in 12 observations with the Tungsten light and a maximum water temperature of 11°C. 43 animals faced the light to 72 that faced away from it; and 48 were counted in the near half to 94 in the far half of the aquarium.

8. With an intense white light at each end of the aquarium the animals tend towards the less intense; if neither light be of great intensity, perhaps not reaching a certain optimum, the animals tend towards the more intense.

9. Reaction to pure red light is the same (though, perhaps, more marked) as to the white; i.e., 225 animals faced the light, in one experiment, to 46 that faced away from the light; and 221 were in the near half of the aquarium to 49 in the distant half.

10. Reactions to blue light are like those to red, but not so marked.

11. The attraction of green light is more marked than the blue but less marked than the red.

12. A small spot of white light from a micro-electric torch produced no effect when thrown on various parts of the body.

13. The animals responded promptly to a beam of sunlight, thrown on various parts of the body, either from above or below, by a small mirror; though if the mirror threw a beam of 5 mm. or less there was little or no response.

14. Animals experimented upon in their native pond, under as natural conditions as could be provided, gave essentially the same responses as described above to sunlight and to an acetylene light at night.

54. Reaction of the whip-tail scorpion to light. BRADLEY M. PATTEN, Laboratory of Histology and Embryology, School of Medicine, Western Reserve University.

The responses of whip-tail scorpions to light were studied with a view to establishing quantitatively, certain characteristic reactions. No attempt was made to treat exhaustively all phases of their behavior under the influence of light. The object was rather to obtain such reaction measurements as would best serve as a basis of comparison for subsequent work directed toward determining the relative effectiveness of the various parts of their complex photoreceptive mechanism.

Reactions to photic stimuli of known intensities were recorded in terms of the induced angular deflections from an initial direction of locomotion. The results obtained may be summarized as follows:

1. The threshold for the kinetic effect of light was at about 0.16 candle meters. The response was clearly negative to all intensities which induced locomotion. Up to an intensity of 1 candle meter the amplitude of the reaction increased rapidly. In the intensities above 1 candle meter the increase in deflection was much more gradual.

2. When started heading away from the source, in a horizontal beam of light of 120 candle meters, animals continued to move along the path of the rays. In 40 trails the average was within 0.6 of a degree of the central ray of the beam.

3. When subjected to a light of 120 candle meters acting on them from the side, the scorpions turned and moved away from the light. The average deflection was 65.8 degrees.

4. When subjected to balanced, opposed lights each delivering an illumination of 120 candle meters, the average trail was within 3.7 degrees of the norm to the line connecting the sources of light.

5. When started directly toward a light giving an illumination of 120 candle meters, the scorpions turned and moved away from the source. The average deflection was 140.4 degrees.

6. Unilateral elimination of any part of the photoreceptive mechanism caused an unbalancing of subsequent reactions. The extent of the unbalance was proportional to the extent of the interference with the receptors.

With regard to the method of orientation these results point to the conclusion that the negative reactions of the whip-tail scorpion to light, depend on a tendency on the part of the animal to attain and maintain bilateral balance of stimulation. Moreover, there are, in this form, no indications that rapid changes of light intensity are necessary to the attainment of orientation. There would seem to be no doubt that light of constant intensity acts as a stimulus. It is apparent, also, that the stimulating effect of light of relatively constant intensity is a prime factor in bringing about and maintaining orientation.

55. *The effect of light and dark upon the eye of Prorhynchus applanatus kennel.* W. A. KEPNER and A. M. FOSHEE, University of Virginia.

1. Stimulation by light results in a contraction of the accessory cell or pigment-cell.

In sustained darkness the cytoplasmic lamellae of the pigment-cell open up or move apart, resulting in the expansion of the cell.

2. The three cytoplasmic regions of the retinula or visual cell show more or less marked changes in response to light and darkness. The nucleus-bearing part of the visual cell is somewhat widened in the dark. The refractive, middle segment-analogous to an ellipsoid of a vertebrate retinula—disappears in continuous illumination and is most conspicuous in eyes that have been subjected to optimum illumination. The rhabdome in light adapted material is a rounded cone-shaped body, while in dark adapted eyes it is an elongated trough-like structure with its long dimension directed parallel to the axis of the body of the animal.

3. Despite the analogy that is apparent between the structure of the retinula of a vertebrate and that of *Prorhynchus*, there is no analogy in functional changes shown. In the former it is the myoid that most markedly changes form, in the latter it the rhabdome is most conspicuously modified in response to light and darkness.

56. *Experimental control of endomixis in paramecium.* R. T. YOUNG, University of North Dakota.

The nuclear phenomena in several lines of *Paramecium* have been observed for a six months' period, during which time several endomixes occurred. The frequency of their occurrence, as measured by the number of generations ensuing between two, may be increased in various ways—by the use of old culture medium, and increase of temperature. Attempts to induce endomixis by the use of ammonia, alcohol, strychnine, nicotine and urea were unavailing. Endomixis is a normal regulatory process in *Paramecium*, not however necessarily associated with depression periods.

57. *Orientation to light in planaria n. sp. and the function of the eyes.* W. H. TALIAFERRO, Johns Hopkins University, (Introduced by Dr. S. O. Mast).

This work is an attempt to correlate an experimental study of the organs involved in orientation to light in *Planaria n. sp.* with their histological structure. In the present study we are interested primarily in the eyes.

All operations were performed upon animals anesthetized with carbon dioxide, with the aid of a knife constructed from fragments of safety razor blades. By means of such technique entire or parts of an eye can be removed. In all cases involving operations, after the behavior of an individual was observed, it was fixed and sectioned in order to ascertain the extent of the injury. The records of only those specimens which showed no injury to adjacent organs were used.

The eye of *Planaria n. sp.* is a typical tricladian eye. It consists of a pigment cup with its mouth opening laterally, anteriorly, and slightly dorsally. This pigment cup has its cavity filled with numerous rhabdomes which are connected by nerve processes with the central nervous system.

Normal specimens of this planarian orient fairly precisely. They are negative and when laterally stimulated turn directly away from the source of light. When, however, a specimen has proceeded a short distance directly away from the light it tends to wander to the right or left. If, in this wandering it turns far enough to allow the rays of light to enter the pigment cup, it suddenly re-orient, suggesting strongly that once the animal is oriented it receives no stimulation unless it leaves the path of orientation. This is opposed to the "continuous action" theory of orientation. During the whole process of orientation the specimen at irregular intervals twists its anterior end so that the ventral side tends to be placed dorsally. This twisting reflex apparently plays no part in normal orientation but has a very definite function in the orientation of forms with one eye removed.

Animals with both eyes removed (without disturbing adjacent organs, such as the nervous system) show absolutely no orientation to light, but they still respond to change of illumination and come to rest in the area of least intensity. From this we may conclude that the directive stimulation of light is received through the eyes.

Animals with one eye removed travel about essentially like normal specimens. There is no evidence whatever of circus movements. If such specimens are stimulated with a horizontal beam of light upon the normal side they turn directly away from the light as do the normal specimens. If the light strikes the "blind" side they do not turn unless in wandering from the path of orientation toward the "blind" side, they swerve enough to cause the light to enter the pigment cup of the remaining eye, or unless during the 'twisting' reflex they twist the head enough to allow the light to enter the eye, or, finally, unless the source of light is raised to an almost vertical position so that again the light enters the pigment cup of the eye. If any one of these conditions is fulfilled they turn directly away from the light, i.e., toward the side having the functional eye. This demonstrates that if the light strikes certain parts of the eye the animal turns toward the stimulated eye, whereas if the light strikes other parts the animal turns away from the stimulated eye.

A number of experiments were carried out to determine the exact extent of these two localized sensory regions. In these experiments it was found that if a beam of light strikes the rhabdomes that lie on the ventral or dorsal lips or the posterior part of the pigment cup the animal turns *toward* the stimulated eye. On the other hand, if it strikes the rhabdomes of the central cavity or anterior part of the pigment cup the animal turns *away* from the stimulated eye.

The above experiments are of further value in that they show that the rhabdomes are not stimulated when the pigment cup lies between them and the source of light. The pigment cups are so placed that once an animal is oriented in relation to a horizontal beam of light, none of it can stimulate any of the rhabdomes unless it does pass through the pigment cup. Since this is so it seems to support the conclusion that once an animal is oriented it receives no stimulation until it leaves the path of orientation sufficiently to allow the light to enter the mouth of the pigment cup.

In a number of animals the posterior part of the eye was removed thus exposing the central rhabdomes to light from behind. These rhabdomes are the ones that in normal orientation cause the animal to turn away from the stimulated eye. Such specimens, however, orient in the same manner as normal ones and proceed away from the light in the same manner although these central rhabdomes are constantly exposed to the light. In another series of experiments the eyes of animals, with both eyes removed, were allowed to partially regenerate. The reactions to light of these specimens were tested at short intervals, and as soon as a given specimen oriented it was fixed and the histology of the eye studied in order to ascertain those parts of the eye necessary for orientation. The results obtained in these experiments show that orientation may occur as soon as a few rhabdomes are formed and that it bears no relation to the position or extent of development of the pigment cup. The results even suggest that the pigment cup is not necessary at all for orientation.

We must conclude therefore that the pigment cup has no function of localizing the otherwise general light stimulation as suggested by Hesse ('97). In other words that the directive stimulation of light does not depend upon the differential shading of the rhabdomes by the pigment cup. And we must also conclude that the light can stimulate a given rhabdome only when it passes through in a certain direction, viz., through some structurally defined axis.

A study of the histology of the eye not only supports the last contention but defines the axis along which the light must pass. By making reconstructions of the eye it was found that all of the rhabdomes in a given localized sensory region are so placed that their longitudinal axes always coincide with the direction of the rays of light that normally stimulate this given region. The rhabdome itself shows an optically denser region in its outer end as described in *Prorhynchus applanatus* by Kepner and Taliaferro ('16). This region, because of its shape and density, must have some effect upon the rays of light if they pass through the longitudinal axis, which it cannot have if they pass through in any other direction.

To sum up, the following statements may be made. The directive stimulation of light in *Planaria n. sp.* is received through the eyes. The sensory part of these organs is composed of rhabdomes. These are so arranged as to form two sensory regions in the eye. Stimulation of one of these regions causes the animal to turn away from, and stimulation of the other region causes it to turn toward, the stimulated eye. The rhabdomes of each of these regions are so placed that the light which normally stimulates a given region has to pass through the longitudinal axis of the rhabdomes. If, however, the normal course of events is altered (by removing part of the pigment cup, etc.) and the light is allowed to pass through some other axis of the rhabdomes, the latter receive no stimulation. As to the function of the pigment cup, none but negative evidence has as yet been found.

58. *Sense of taste in Nereis virens.* ALFRED O. GROSS, Bowdoin College.

The food of the adults of *Nereis virens* was investigated by an examination of specimens secured in various situations of several localities. In all cases it was found to be made up almost entirely of vegetable matter—animal life as a food was merely incidental.

Nereis exhibits no marked responsiveness to food material, but was very strongly negatively chemotrophic to even the weak solutions of acids, alkalies and hydroxides in sea water. The worms were tested by placing them on a narrow paraffin dam, built across a rubber developing tray, in such a way that they were free to withdraw into the sea water on one side or crawl into the sea water containing the stimulating substance on the other side. The average time required for each of a series of 24 animals to withdraw from various strengths of solutions was determined under conditions controlled for the factors of light and temperature. The cirri, palps and tentacles were then cut off from all of the series excepting those used as a control. Tests

made after the animals had fully recovered from the operation showed the worms to have a decided increase in the time required to withdraw from the stimulating solution. After the organs regenerated on the operated animals the reaction time was practically equal to that of the normal individuals. A large number of experiments were conducted in which only one or various combinations of organs were removed. In all cases the average time for the worms to withdraw was determined before and after the organs were destroyed and again when the parts had regenerated.

The chemical sense was found to be localized to definite regions, and apparently depends on the presence of clusters of sense cells.

59. *The influence of the marginal sense organs on functional activity in Cassiopea xamachana Bigelow.* LEWIS R. CARY, Princeton University.

A. THE INFLUENCE OF THE SENSE ORGANS ON THE RATE OF REGENERATION

Previous studies have demonstrated a marked influence of the sense organs on the rate of regeneration in *Cassiopea*, when halves of the same disk are used for comparison. As this influence is most marked in the earlier stages of regeneration several series of disks were prepared by first separating each disk into halves and then removing the sense organs from one of each pair of half-disks at varying intervals after the first operation. If the sense organs were removed within less than twenty-four hours after the first operation the regeneration would be most rapid from the half upon which the sense organs remained. When the sense organs were allowed to remain on both half-disks for twenty-six or more hours before the second operation the amount of regeneration was equal for both halves.

B. THE INFLUENCE OF THE SENSE ORGANS ON THE LOSS OF WEIGHT IN STARVING DISKS OF CASSIOPEA

When an entire medusa, or the separated disk of *Cassiopea* is starved in sea water from which all food material has been removed by careful filtration, the loss of weight follows a curve that has the mathematical formula $y = W(1-a)^x$, in which W is the original weight, x the number of days of starvation and a a constant the "coefficient of negative metabolism." While the value of a in the equation above will differ in experiments involving differing conditions of light, regeneration, density of the water, etc., this formula gives a very close approximation to the observed result in all cases.

In the following experiments the disks were prepared in such a manner that they fell into one of three groups. In the first group one half of each disk retained its sense organs, while these structures had been removed from its mate (active and inactive series). In the second group one half-disk retained its sense organs, while after the removal

of these structures from the other half its muscles were activated by a circuit wave of contraction started by an induction shock, (active and activated series). In the third group activated and inactive half-disks were compared.

The greatest loss of weight was shown by the half-disks with sense organs, a considerably smaller loss by the activated specimens and the smallest loss by the inactive group. In every instance these results follow very closely those obtained when the rate of regeneration was used as the standard of measurement.

A summary of the results of the entire series of experiments is shown in the following tables.

TABLE 1
Loss of weight in active and inactive half-disks

DAYS AFTER OPERATION	WEIGHT OF HALF WITH SENSE ORGANS	WEIGHT OF HALF WITHOUT SENSE ORGANS
0	100.00	100.00
1	76.84	81.81
2	66.72	71.27
3	58.54	64.09
4	55.27	55.41

TABLE 2
Loss of weight in active and activated half-disks

DAYS AFTER OPERATION	WEIGHT OF HALF WITH SENSE ORGANS	WEIGHT OF ACTIVATED HALF
0	100.00	100.00
1	76.29	79.41
2	67.18	70.58
3	59.76	61.99
4	55.88	57.18

In both these experiments the weight is reduced to 100 grams for the original weight of the series. The comparison of the loss of the activated disks with the inactive disks can be made by using the right hand columns in tables 1 and 2.

In the experiments where active and activated disks were used the pulsation rate of the latter was on the average about three times that of the former at the beginning of any experiment. In the later stages the difference in the pulsation rate became greater, until at the close of the first twenty-four hours the activated halves were pulsating ten times as fast as the halves the pulsation of which were under the control of the sense organs. This change in the reactivates of pulsation comes about both through a decline in the rate of the active half-disks and in increase in the rate of the activated halves.

Simultaneous kymograph records of the pulsations of the halves of the same disk, one with its sense organs and the other with an entrapped wave of contraction in its subumbrella muscles, showed that

when a like series of cuts were made in the subumbrella tissues of each half the amplitudes of the contractions were equal so that pulsation rate affords a true measure of the muscular activity of each half-disk.

C. THE INFLUENCE OF THE SENSE ORGANS ON THE TOTAL METABOLISM OF *CASSIOPEA*, AS MEASURED BY CARBON DIOXIDE PRODUCTION

Since in experiments where either the rate of regeneration or the loss of weight during starvation had been used as the standard of measurement it had been shown that there must be some other more important factor involved than that of muscular activity, the total metabolism of half-disks prepared as in the former experiments was determined. Here again the muscular activity was the most apparent difference between the half disks.

To measure the total metabolism (respiratory activity) the half-disks were put separately into jars of fresh sea water, each containing 1200 cc., provided with a clamped top so that none of the gases could escape. After equal intervals of time each half of any pair of disks was removed from its jar and the amount of CO_2 that had been given off determined by ascertaining the change in the hydrogen ion concentration that had taken place in the sea water.

Under these conditions the pulsation rate of any half disk first increased for a short time under the stimulating effects of the increase of hydrogen ions. There then followed a progressive decrease in the pulsation rate until finally the disks became quiescent from the anaesthetic effect of the CO_2 , which they had themselves given off. In nearly all instances the activated half-disk was the first to succumb to the CO_2 , and the decline in its rate of pulsation was quite steady after the preliminary rise in response to the first increase in acidity. The half-disk with sense organs was always very erratic in its pulsations and after a long quiescent period would frequently, just before complete anaesthesia, pulsate for a few moments at a rate several times that shown under the influence of the first CO_2 stimulation.

When the jars in which these experiments were carried out were allowed to remain in daylight the disks would continue to pulsate for several days, as the CO_2 would be for the most part used up in the metabolism of the symbiotic algae which are so abundant in the tissues of *Cassiopea*.

The normal reaction of the sea water at Tortugas varies from PH 8.1 to PH 8.2 ($\text{CH } 8 \times 10^{-9}$ to 6.3×10^{-9}). After a half-disk had been kept in one of the closed jars for several hours the reaction would be changed to PH 7.9 (approximately) after which the change was very slow, anesthesia soon following. Using the same volume of sea water it was found that the addition of from 4 to 5 cc. of pure CO_2 would bring about the same change in the reaction of the water as that caused by the respiration of the half medusa disk.

The amount of CO_2 given off from the active or activated half-disks seldom varied more than 0.1 of the PH unit, and in most experiments

the more slowly pulsating half with its sense organs had the higher rate of metabolism. The muscular activity, on the other hand, was in the proportion of one to three at the start of the experiment, and rose to about one to ten during the experiment, the activated half-disk having always the higher rate.

60. *The relation between the hydrogen ion concentration of sperm suspensions and their fertilizing power.* EDWIN J. COHN, University of Chicago. (Introduced by Frank R. Lillie.)

The activity of the spermatozoa of *Arbacia* is a function of the hydrogen ion concentration. In sea water less alkaline than 0.5×10^{-7} sperm, are apparently non-motile. In such a medium, however, they live very much longer than in natural sea water, as measured (a) by their power to fertilize ripe eggs of the same species and, (b) by the dissolution of their protoplasm. In sea water more alkaline than the ocean (i.e., H.I.C. of $.08 \times 10^{-7}$) the life of the sperm is shortened.

The often repeated observation that more concentrated sperm suspensions live longer, is also attributable for the most part, to the greater hydrogen ion concentrations in the denser suspensions. For, the more concentrated the suspension, the more carbon dioxide is at first produced by the sperm themselves. The ionization of the carbonic acid thus formed increases the hydrogen ion concentration of the suspension, and decreases the activity of the sperm. Measurements of the total carbon dioxide production of sperm suspensions of varying concentration show that sperm that live twenty-four hours produce no more carbon dioxide than sperm that live for only four hours. Using the carbon dioxide production as the criterion, we must conclude that the activity of the sperm is limited. Therefore, sperm that have been in alkaline sea water have a greater fertilizing power, but for a shorter time, than sperm that have been in unmodified sea water, while sperm that have been in acidified sea water, where they have been relatively inactive, retain their power to fertilize for a much longer period.

61. *Experimental study of ageing eggs and sperm and of their development.*

A. J. GOLDFARB, College of the City of New York.

For the last two years experiments were made with the sea-urchin eggs of *Toxopneustes* and *Hippanoe* of the Florida coast and *Arbacia* of the Massachusetts coast.

1. The first series of experiments were made with a view towards obtaining experimental conditions that were optimum and that gave the least variability for freshly removed eggs and sperm, from freshly collected sea urchins.

2. Surprisingly large differences were observed among the different females particularly *Toxopneustes* and *Hippanoe*. Such individual variability involved (a) size of eggs; (b) presence or absence of jelly; (c) rate of membrane formation; (d) character of membrane; (e) rate and total cleavage; (f) character of cleavage.

3. By means of one or more of these criteria it was possible to grade the different freshly collected females according to the physiologic

condition of their eggs. Eggs of similar physiologic condition showed a minimum variability and the highest correlation with respect to these six categories.

4. When eggs and sperm were removed from their respective bodies and kept under optimum laboratory conditions, the same physiologic changes which had begun within the bodies of the urchins continued outside of the body.

5. With increasing age outside of the body, the eggs showed progressive changes in (a) size; (b) loss of jelly; (c) retardation of membrane formation; (d) decreased extension of membrane formation; (e) decreased percentage of cleavage, (f) decreased rate of cleavage.

6. These changes were closely proportional to the time after removal of the eggs from the urchins. Knowing the age one could predict the physiological and developmental changes in the eggs; and vice versa knowing the condition of the eggs one could estimate their age.

7. The physiologic and developmental changes enumerated above were correlated.

8. Further studies made clear in what regards ageing eggs fertilized by fresh sperm, differed from ageing sperm by fresh eggs, and these in turn from eggs and sperm that aged synchronously. This also made possible the determination of the maximum longevity of eggs and of sperm.

9. Other changes consequent upon ageing of eggs suggested the nature of the chemico-physical agencies involved in the ageing process, namely, (a) agglutination and fusion of eggs; (b) tendency to develop irregularly; (c) tendency of blastomeres to separate.

10. These changes suggested that the excess free HO ions in sea water was one agency and probably a very important one in causing the dissolution of the jelly, the changes in the permeability of the cortical layer of the eggs, the changes in size and all the other changes mentioned, that follow upon long exposure to the free HO ions.

11. If these ions are involved in the ageing process, one should be able to age eggs precociously with hyperalkaline sea water, and one should be able to retard their ageing with sea water made neutral. These experiments were repeatedly made. Eggs were made to age precociously and show all the physiologic and developmental changes enumerated above with hyperalkaline sea water, or their ageing was retarded more surprisingly by removing excess HO ions.

12. The longevity of the eggs were increased either by reduction of respiration by KCN as proposed by Loeb, Lyon and others, or by eliminating the effect of the free HO ions of sea water.

13. Other changes involving the metabolism of the eggs and sperm will be reported elsewhere.

62. *The consumption of oxygen during the development of fundulus heteroclitus.* GEORGE G. SCOTT, College of the City of New York, and WM. E. KELLICOTT, Goucher College.

The energy for development is derived from the oxidation of nutrient materials. This energy is partly consumed in the *processes* of develop-

ment, and is partly represented by the chemical structure of the differentiated substances of the organs and tissues of the embryo as contrasted with that of the yolk-substance. The rate at which oxygen is consumed is an index of the rate at which all of these changes occur.

During the summer of 1916 the authors, working jointly at the Bureau of Fisheries and the Marine Biological Laboratory, Woods Hole, carried on a series of observations on the rate of oxygen consumption during the entire developmental period of *Fundulus heteroclitus*, from fertilization until ten days after hatching. Seven distinct series including about 11,000 eggs were observed, some of which were combined and others divided during the period of observation. Methods of study were improved during the course of the experiments, but some series were treated uniformly throughout.

Known numbers of eggs were placed in air-tight containers, immersed in running sea-water. Temperatures thus varied only slowly between limits of 19.4 and 21.4°C. The water covering the eggs was renewed at appropriate intervals, samples being taken at the beginning and end of each interval. The amount of oxygen present in these samples was determined by the Winkler method. Altogether about 700 determinations were made. From these determinations the amount of oxygen consumed was calculated and expressed in cubic centimeters consumed per thousand eggs per hour. While this preliminary report is based upon the study of only a part of our data and is therefore subject to modification in details, certain essential facts may be stated.

The amount of water allowed per egg per hour was varied considerably in different series. Two series were kept enclosed throughout the entire period to hatching, save for the brief intervals of sampling; others were sealed for observation only a few hours at a time and during the intervals between these periods were kept in large flat dishes, loosely covered.

The duration of the period before hatching varies, not only with temperature, but particularly with the amount of oxygen actually available. The total amount of oxygen consumed and the relative rates of its consumption during different phases of development do not vary materially, so long as a certain minimum is not passed, whether the average age at hatching be 17 days (normal at the temperature used) or more than 40 days.

The more striking features in the varying rate of oxygen consumption are best shown in graphic form [see chart].

During the early stages of development—cleavage, embryo-formation, etc.—the hourly consumption of oxygen is less than 0.10 cc. per thousand eggs. A marked rise in the rate occurs at the time the circulation is established, after which it remains practically constant, though with an upward trend, until shortly before the time of hatching.

Approximately 80 cc. of oxygen are consumed by 1000 eggs up to this time. At hatching and thereafter the increased energy demands incident to muscular activity are reflected in a very great increase in oxygen consumption. During the hours immediately after hatching the

rate rises to about 0.7 cc. per hour per thousand, which increases steadily to more than 1.75 cc. about six days after hatching. By that time the yolk is almost wholly absorbed and the rate of consumption falls off rapidly, in the absence of a food supply.

Determinations were made of the amount of protoplasm and of yolk present at the commencement of development and of the weight of the embryo after hatching. During early cleavage, when the amount of protoplasm can be accurately determined, a thousand eggs contain 0.12 cc. protoplasm and 2.65 cc. yolk-material, a total weight of approximately 2.9 grams. At six days after hatching 1000 larvae weigh approximately 1.8 grams, nearly all of which consists of differentiated tissues. There is thus a loss of about 1.1 grams or 38 per cent of the initial weight of protoplasm and yolk. In effecting this transformation roughly 270 cc. of oxygen are consumed, that is, a little more than 0.25 cc. per egg.

63. *A study of broodiness in the Rhode Island red breed of domestic fowl.*

H. D. GOODALE, Massachusetts Agricultural Experiment Station.
(To be read by title.)

In broody races of domestic fowl, such as the Rhode Island Reds, a pullet lays a variable number of eggs and then exhibits a desire to incubate them. When poultry is kept for eggs it is the practice of poultrymen to prevent the hen from gratifying her desire. Under such circumstances manifestations of broodiness disappear after a few days and after a period of variable length the hen begins laying again. This time only a comparatively few eggs are produced before the manifestations of broodiness reappear with consequent cessation of production. This cycle of alternate periods of production and non-production is repeated about once in 35 days until late summer or fall when production ceases until sometime in the winter or spring, when they are resumed. The present study is concerned with the relation of the various parts of the cycle to each other, their distributions in time and the effect of broodiness on egg production.

The results of most general interest are as follows: The length of the period before the first broody period appears may vary from a month up to two or even more years, while a very small per cent have never exhibited signs of broodiness. Ninety-five per cent, however, of the birds go broody before July 1 of their pullet year. The number of broody periods depends in part on the data of the first broody period and in part on the time the bird stops laying in the fall and may vary from one to eleven times during the first year. In the second year, broody periods begin as soon as the bird lays a comparatively few eggs.

The effect of broodiness on egg production is very marked, for if the rate of production (eggs divided by time in days) before the first broody period be compared with that for the remainder of the year, it is found that, on the average, production drops about 40 per cent. Other things being equal, then, broodiness lowers annual production

very markedly in this breed. The statistical constants for other phases of broodiness have been calculated and will be published as soon as possible.

Clear evidence that it will be possible to separate a strain of non-broody birds of this breed has been obtained, if indeed the strain has not already been established.

64. *The vitality of cysts of Didinium nasutum.* S. O. MAST. Johns Hopkins University.

Didinia cysts which had formed about the middle of June, 1910, were put into a 10 cc. vial May 31, 1911. The vial was then sealed air-tight. Cysts were taken from this vial from time to time, usually once a year, and tested for vitality by adding them to vigorous cultures of paramecia. The last test was made in March, 1915, nearly five years after the cysts had formed. In all of these tests some active didinia were obtained. Only a very small per cent of the cysts developed in any of the tests and in the last one this percentage seemed to be considerably smaller than in the others. In this test only a very few didinia came out but these developed rapidly and produced a very vigorous race which was normal in every respect. Didinia cysts can, therefore, retain vitality for at least five years. All of the cysts were used in the last test so that it was impossible to carry the experiment further.

Drying in ordinary atmospheric conditions does not destroy the cysts. In fact, there is some evidence which indicates that they would live longer dry than in a solution.

65. *The reactions of Pelomyxa Carolinensis Wilson to food.* By W. A. KEPNER and J. G. EDWARDS, (University of Virginia).

These multinucleated rhizopods feed upon both animals and plants. Their food consists, so far as our observations go, of nematodes, ciliates, flagellates, diatoms and desmids. They show a preference for ciliates and flagellates, being very fond of *Chilomonas paramecium* and *Paramecium caudatum*.

There are three factors involved when all the effective stimulation of *Pelomyxa* to food-reaction are considered. These are (a) contact with bodies, (b) play of currents of water upon the rhizopods, and (c) chemical agencies, such as oxygen given off by green plants and carbon dioxide given off by bacteria and animals. Perhaps the most potent of these factors in determining which of the two types of food-reactions will be carried out is that presented by the currents of water set up by the play of cilia or flagella of animals and plants.

In reacting to objects of food, that give off currents in water and are able to dart away, the *Pelomyxa* avoids producing on the prey an effective stimulus by making a wide detour about the apparently quiet specimen. This represents one of the two types of food-reaction of *Pelomyxa* that we have observed.

In reacting to objects that are stationary and give off no currents in the water, the *Pelomyxa* applies itself intimately to the surface of

the object as it is being ingested. This represents the second type of Pelomyxa's food-reaction.

The details of the type of reaction involved when the Pelomyxa is reacting to an object, that is lashing its cilia or flagella and but for thus disturbing the water is otherwise quiet, are highly variable. Its reaction to such an object is modified to meet the conditions presented by each situation. For example, if Paramecium or Chilomonas lie in the open, it is surrounded on all sides by the advancing Pelomyxa; if the Paramecium or Chilomonas lie by a desmid, a pseudopod will be thrown up in such manner as to enclose the prey between the pseudopod and the desmid. In this manner the prey becomes entrapped between the advancing pseudopod of the rhizopod and the desmid. In this trap it is held until the pseudopod bends back upon the prey and encircles it on all sides leaving the desmid, which has been used as an aid in catching the protozoon, behind when the food vacuole has been completed and the animal is ingested.

The complex reaction to a highly motile, but apparently quiet protozoon may be arrested and reversed, if the prey escape; or it may advance until the acquired impetus of a sustained reaction has been spent, in some cases resulting in a fully formed food-vacuole.

Pelomyxa, when first stimulated by Paramecia that are actively darting about, reacts by sending out a flood of cytoplasm at the point of collision with the ciliate. Eventually, however, the reaction of the hungry rhizopod to the Paramecia is changed, so that it becomes less vigorous and, one could almost say, more cautious. After fifteen minutes of bombardment by the Paramecia the Pelomyxa ceases to present this futile flooding of cytoplasm and settles down to the more deliberate reactions characteristic of it when it feeds upon ciliates.

The details of the manner in which each peculiar situation is met when Pelomyxa reacts to food are so varied as that no theory yet advanced for the explanation of pseudopod-formation or food-reaction promises to satisfactorily explain them.

66. *The significance of conjugation and encystment in Didinium nasutum.*

S. O. MAST, Johns Hopkins University.

In April, 1910, a pedigree culture consisting of two groups of four lines of didinia was isolated. In one of the groups conjugation occurred at the time of isolation in the other group it did not. From the offspring of the latter other groups of lines were from time to time isolated, some immediately after conjugation, others immediately after encystment, and still others without either conjugation or encystment. These various groups, in isolation cultures, were carried along in parallel series, so that at any given time the number of generations which had been produced since conjugation and encystment had occurred in the different groups differed, in some instances but little in others very much. Thus the cultures were continued, with certain intermissions, until May, 1914.

At the close of the experiment there had been produced in one of the groups an average of 1646 generations without conjugation, and earlier

the same group of lines had passed through 1035 generations without encystment. The stock became very weak toward the close but it did not die out, and, of course, it is not known how much longer it would have survived. The fact that it continued so long without conjugation or encystment seems to indicate that these processes are not necessary for continued existence.

The specimens in all of the groups were treated as nearly alike as possible. They were all fed on paramecia from the same jars, subjected to the same temperature and kept in the same sort of solution. Consequently since all originated in the same individual they differed at any given time, merely in the number of generations produced since conjugation or encystment had occurred. If, therefore, these processes cause an increase in the rate of fission as Calkins maintains, or if conjugation causes a decrease in the rate of fission and an increase in death-rate and in the variability in the rate of fission as Jennings maintains, it should become evident in a comparative study of the characteristics of the individuals found in the different groups at any given time. Such a study shows, however, that while the fission-rate the death-rate and the variation in the rate of fission varied greatly at different times, owing largely to changes in temperature and variations in the culture solution, they were essentially the same in all of the groups at any given time throughout the entire experiment. This seems to show that neither conjugation nor encystment in *Didinium* appreciably affects the vigor of the stock or the variability in the rate of fission. It seems to prove that these processes are not rejuvenating processes, at any rate not in the sense in which Calkins has used this term: namely, that it consists in reorganization in which accumulated waste materials are eliminated.

This conclusion is, moreover, supported by the fact that toward the close of the experiment when the stock was very weak it was almost impossible to induce encystment, and by the fact that conjugation which occurred very freely in mass cultures of this weak stock produced no improvement whatever, in fact, these weakened didinia in mass cultures where conjugation occurred abundantly, died out much more freely than they did in isolation cultures where conjugation did not occur at all. If the loss of vigor in the stock is due to an accumulation of waste and if conjugation and encystment serve to eliminate this waste as Calkins maintains why was there no improvement in the weakened stock of didinia in which both of these processes occurred? And why was there no improvement if conjugation causes an increase in variation which results in improvement in adaptation to existing conditions as Jennings maintains? There seems to be no answer to the first of these questions but the second may be dealt with as follows:

Jennings assumes that the production of favorable characters is due to the union of nuclear substances which differ in potency. If, therefore, the nuclear potency of the conjugants is the same one would not, in accord with Jennings' contention, expect any favorable effect.

The didinia used in the experiments described in the preceding pages were very closely related. It may, consequently be maintained that the failure to obtain any effect by conjugation was due to the similarity of the nuclear potency of the conjugants. If this is true, it is obvious that our results do not militate against the contentions of Jennings as set forth above.

67. *Some distributional problems of Okefinokee swamp.* A. H. WRIGHT, Cornell University.

Many of the forms of the dry open sandy fields or pine forests of southeastern United States were absent on the Okefinokee Swamp Islands, e.g., six species of snakes and the gopher turtle. The last form suggests the swamp's influence on subterranean mammals of southeastern Georgia. Some of these in their avoidance of the swamp and in their enveloping of it often have their range limits quite abruptly marked by the swamp, e.g., three species of gophers, three short-tailed shrews, and the pine mouse. The swamp is the common source of the Atlantic coastal stream, the St. Mary's and the Gulf affluent, the Suwannee. We have no collections from the lower courses of each of these and cannot now discuss this factor. We had expected to find fixed peculiar stable races or subspecies because of the isolated nature of some of the islands but segregation has not yet placed a definite local stamp on the forms within the swamp. With the dry land forms it is a barrier but for others it is rather a melting pot for many of the supposed cardinal characters of distinction. Or it may represent the inherent variation possible in one limited geographical region, not what might occur in an extensive or expansive stretch of territory, e.g., pilot snakes with the temporal scutellation of seven supposed different forms, the overlapping of scale rows and ocular formulae of the DeKay's and red-bellied snakes, the presence of *Osceola* and *Lamprokeltis* characters in one specimen, etc., etc. Or the swamp may be interpreted as a meeting ground of two or three faunas or elements, e.g., three types of racoons, one from the north, one from Florida, and one from the west, or two bears, one the Floridan, the other the Louisianian with northern tendencies. Many of the introduced forms of the Atlantic seaboard to the immediate east, like the house mouse, black rat, roof rat and English sparrow are absent. The largest mammals of southeastern United States are there in great abundance and these carnivores may account for the scarcity of small mammals.

68. *A means of transmitting the fowl nematode, Heterakis papillosa bloch.*

By JAMES E. ACKERT, Kansas State Agricultural College.

It has been found recently that the fowl nematode, *Heterakis papillosa* Bloch, may be transmitted to chickens by the feeding of dung earthworms, *Helodrilus parvus* (Eisen), taken from poultry yards in which the fowls were heavily infested with *H. papillosa*. The chickens were reared from time of hatching under controlled conditions, and feedings of *H. parvus* were made on three different occasions, infec-

tions of the fowls resulting each time. Other chickens kept in the same enclosure, but not fed experimentally, were free from nematodes. Whether or not these are cases of parasitism or of mere association remains to be determined.

69. *Further studies on changes in Thelia bimaculata brought about by insect parasites.* (Illustrated with lantern.) S. I. KORNHAUSER.

At a previous meeting of this society (Columbus, 1915), the author discussed various modifications of the Membracid, *Thelia bimaculata*, produced by internal insect parasites (then unidentified). The most striking change occurs in the male which, when parasitized early in its ontogeny, assumes normal female coloration. The pronotum of the male is normally dark brown with a bright orange-yellow vitta on each side; the female is gray, the vitta being only slightly visible. In parasitized males the hypodermal yellow pigment disappears from the vitta, the punctures become dark with melanic pigment, and the rest of the pronotum loses its uniform brown color; the melanin being restricted to the punctures and the greenish-yellow hypodermal pigment showing through the chitin between the punctures. Thus true female color is established by the loss of male characteristics and the gain of female characteristics. A series of stages was gotten showing the gradual disappearance of the yellow and the assumption of melanin in the vitta of various males, the degree of change depending on the state of development of the parasites in the fifth instar of *Thelia* previous to the final moult. No change in color takes place in parasitized females.

During the past year an abundance of material has been collected and a more complete study made of the changes in both sexes, nymphs as well as adults. The parasites have been reared and shown to be a new species of the genus *Aphelopus*, one of the Dryinidae. This hymenopteron lays its egg apparently in the nymph of *Thelia*, where it undergoes polyembryonic development passing through a complicated hypermetamorphosis. Fifty to sixty larvae result and become full-grown during the fifth instar of the host, if the egg from which they developed was deposited in a *Thelia* of the first or second instar. In this case the larvae emerge from holes in the ventral side of the nymph, after devouring everything within the chitin of the host. They drop to the ground, burrow and pupate. On the other hand, if the parasites are only partially developed in the fifth instar, the host becomes an adult, but modified accordingly to the degree of development of the parasites. With greater difficulty they may later emerge from the adults.

The males of *Thelia* possess in diploid number twenty-one chromosomes, the largest of which is the X-chromosome. The female has twenty-two chromosomes, including two large X-chromosomes. These facts were corroborated during the past summer by observations on the developing external genitalia, the size relations in the chromosomes in these soma cells being exactly those previously found in the spermatogonia and oögonia.

Nymphs of the second instar show external sex differences through the form of the segments which produce the external genitalia. These differences become more marked in the third instar, more so in the fourth, and still more in the fifth. The testes develop greatly but the ovaries remain small even until the fifth instar, although the size of the female as a whole is greater than that of the male. If the individual is parasitized early, the growth of the external genitalia is retarded in either sex; so that those of the fifth instar resemble in form and size those of the normal fourth instar. Neither sex changes toward the opposite sex nor toward a neutral form in the genitalia. The size of the abdomen does become greater in the parasitized male.

A comparative study was next made of the sizes of parasitized and normal adult *Thelias*. The results are given in tabular form:

	NUMBER OF INDIVIDUALS	AVERAGE LENGTH OF PRONOTUM	AVERAGE WIDTH SAME
		<i>mm.</i>	<i>mm.</i>
Normal males.....	114	11.55	4.73
Parasitized males.....	127	12.11	4.98
Parasitized males with changed color....	98	12.24	5.03
Parasitized males no change in color....	29	11.68	4.85
Normal females.....	111	13.39	5.41
Parasitized females.....	100	13.11	5.33

Thus parasitized males increase considerably, whereas parasitized females show a slight decrease in size. With the assumption of female color, males tend toward the female in size and form.

This is shown too in all other organs of the body: wing size, size, pattern and color of head; size of proboscis; size of legs; size of endosclerite; size of digestive tube; and size of abdomen, especially the terminal segments. The parasitized female is just a little undersized but normal in form, except that the ventral and terminal plates of the abdomen are soft, non-pigmented, and much like those of the fifth instar. This is the only juvenile character found in parasitized adults.

The parasites effect a reduction in size of the external genitalia of both male and female adults, but neither resembles the opposite sex. In parasitized males with normal color the genitalia may be considerably reduced in size, showing that they are very easily effected.

The changes are not directly due to the state of the primary sex organs; for, in normally colored but parasitized males, the testes may still be quite normal at the time of the final moult. This summer one unique male was found: parasitized, with female coloration; but with one testis intact, slightly undersize but with normal mitoses and spermatozoa. The changes in the secondary sexual characters are doubtlessly to be correlated with changes in nymphal metabolism. The male nymphs grow more rapidly, are smaller in size, darker in color, and are sexually mature when they become adults, the testes filling the greater part of the abdomen. The female nymphs develop more

slowly, become larger, store fat, and when they moult are prepared for the production of food for egg materials. The male is active and has a higher power of oxidation; the female is anabolic to a greater degree. The presence of parasites in the male nymph brings about lower oxidation, storing of fat, retarded rate of development, increased size; and with this change in metabolism comes a change in some of the secondary sexual characters. But changed metabolism is not powerful enough to change the external genitalia, it merely reduces them in size. These organs are laid down early in the ontogeny of the animal and are doubtlessly as old phylogenetically as the sex chromosomes, which make one male and the other female. But such secondary sexual characters as color and form, which have probably arisen later phylogenetically, through sexual selection or otherwise, are altered when a change in metabolism takes place.

70. Some experiments on the transmission of swamp fever by insects.

JOHN W. SCOTT, University of Wyoming.

In 1915 the writer reported at Berkeley an experiment which appeared to prove that swamp fever in horses may be transmitted by the stable-fly, *Stomoxys calcitrans*.

The experiments to be mentioned here required the construction of a second screened cage large enough to hold six horses. Into Cage A three horses in good condition were kept, and two well horses were placed in Cage B. Three available diseased horses were exposed alike in the two cages; these horses were so rotated through the cages that there was always at least one diseased horse in each cage, and no horse remained longer than two successive days in either cage. Stable-flies were raised and kept in Cage A, and Cage B was kept free from flies. Two out of three of the well horses in Cage A contracted the disease, and the well horses in Cage B remained uninfested. It is believed that we have here strong circumstantial evidence that stable-flies were responsible for the infection.

In the next experiment stable-flies were taken from Cage A, confined in fruit jars with a mosquito netting cover, and exposed through the netting to the backs of two horses that were running free in a lot. Early frost in the fall of 1915 cut short this experiment after a few such exposures. However, in about two weeks one horse first gave evidence of developing the disease; the other horse gave no immediate signs of infection though some eight months later he developed a slow-going chronic case. Since we have never had a case develop as the result of horses running together in a lot with diseased horses, the experiment affords additional proof that *Stomoxys calcitrans* may transmit swamp fever.

In the experiments performed during the past summer, all diseased horses were kept in Cage A, and all well horses experimented with were kept in Cage B. Again stable flies were kept in Cage A, and no biting flies allowed in Cage B. One of the experiments was to determine in a rough way the smallest amount of infective blood that would

produce the disease. For this purpose a medium fine hypodermic needle was used to puncture the skin of a diseased horse, and then without drying or washing was used to puncture the skin of a horse in Cage B. This operation was repeated several times, but not more than twice on the same day. The horse used in Cage B developed the disease. This experiment shows that the amount of virus necessary to produce the disease is very small, and that a swarm of flies, as the result of interrupted feeding, could easily convey enough of the blood to produce swamp fever. In a second experiment green headed flies, *Tabanus* (Sp ?), were used; the interrupted feeding method was employed, first allowing the tabanids to bite diseased horses and then a well horse in Cage B. Though we could use only one horse, the result indicates that swamp fever can also be transmitted by these flies. In a third experiment stable-flies were again used. These were exposed in small wire cages to the backs of swamp fever horses; after they had begun to feed they were transferred to the backs of two healthy horses in Cage B. One of these has apparently contracted a mild form of the disease; the other so far has shown no signs of an infection, and since he was used in an experiment in 1915 with negative results he may be immune.

A full account and discussion of the foregoing experiments will appear in a short time. It seems clear:

1. That swamp fever can be transmitted by certain biting insects such as *Stomoxys calcitrans* and probably *Tabanus* (sp ?);

2. That a mechanical transmission of infected blood in extremely small amounts is sufficient to produce the disease.

3. That the insect theory of transmission is sufficient to account for epidemics of swamp fever, accords well with the seasonal distribution of cases, and explains why the worst epidemics as a rule occur during wet seasons, when biting flies are usually most abundant;

4. That these experiments do not forbid the idea that some certain intermediate host is a still more effective means of distributing the virus.

A full explanation cannot be given until we know more about the nature and cause of the disease. It is known that swamp fever is due to one of the filterable viruses; the virus is capable of being propagated in the body of the horse, and so far has not yielded to staining or to bacteriological technique. Whether the virus is capable of self-propagation, or partakes of the nature of a cell product, a sort of pathologic hormone as it were, remains to be answered.

71. *The domestic cat a host of Taenia pisiformis Bloch.* By J. E. ACKERT and A. A. GRANT, Kansas State Agricultural College.

An attempt to infect domestic cats with the common dog tapeworm, *Taenia pisiformis* Bloch, has apparently succeeded. Of ten kittens, reared under controlled conditions and fed cysticerci from the viscera of cottontails, *Sylvilagus floridanus mearnsii*, eight kittens became infected with *T. pisiformis* (two to eight parasites per individual).

None of the controls (four kittens of the same litters as the experimental ones) harbored any tapeworms. The sexually mature specimens obtained are evidently young *T. pisiformis*, being somewhat smaller than the average adult of this species. This is another of the instances in which adult carnivore cestodes are sufficiently generalized to develop in hosts belonging to different families.

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

ARTICLE II

MEMBERSHIP

Section 1. Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary-Treasurer and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary-Treasurer and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary-Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in the Secretary-Treasuryship occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

BY-LAWS

DUES

(1) The annual dues for members, unless remitted or changed by the vote of the Society, shall be seven dollars.

SECRETARY-TREASURER

(2) The duties and privileges of the Secretary-Treasurer shall be as follows:

(a) He shall keep the records and be in charge of the funds of the Society.

(b) At the annual business meeting he shall present a statement to date of the funds of the Society.

(c) Whenever the proper officers of a number of related societies shall have a conference with a view to determining a common time and place for the several annual meetings, he shall act as the delegate or representative of this Society. (See also 4-a.)

(d) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(e) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

AUDITING COMMITTEE

(3) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Secretary-Treasurer at the meeting for which they were appointed.

AFFILIATION WITH THE AMERICAN SOCIETY OF NATURALISTS

(4) Affiliation with the American Society of Naturalists is provided for as follows:

(a) It shall be the policy of the Society to hold its annual meetings in conjunction with the American Society of Naturalists, whenever satisfactory arrangements to do so can be made; and, with this in view, before the time and place for an annual meeting shall have been determined by the Executive Committee, the Secretary-Treasurer shall ascertain from the Secretary of the American Society of Naturalists the time and place for holding the annual meeting of that Society. It shall be the policy of the Society, however, to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. (See also 2-c.)

PROGRAM RULES

(5) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4.

Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary-Treasurer.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. When a member offers more than one paper those following the one designated first shall be placed at the end of the list and shall not be read until all first papers by members shall have been twice called for.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of "introduced" papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Each title sent in for the program must be accompanied by a typewritten abstract of the paper for publication and distribution to members of the Society before the meeting.

HISTORICAL REVIEW

The American Society of Zoölogists, as now organized, is directly descended from the American Morphological Society, the Central Naturalists, the Zoölogical Society of America, and the Eastern and Central Branches of the American Society of Zoölogists. A brief review of the history of these societies is, therefore, of considerable interest.

The material for this account is taken in part from the preface to the List of Members of the American Society of Zoölogists issued in 1906.

AMERICAN MORPHOLOGICAL SOCIETY

During the year 1890 the following circular, which resulted in the formation of the American Morphological Society, was issued:

OCTOBER 16, 1890.

DEAR SIR—During the last ten years there has been a very noticeable increase in the number of persons in this country who are devoting themselves to the study of animal morphology. With the increased enthusiasm which has arisen for such studies, and with the increased facilities for their prosecution which we now possess, it seems probable that the near future will witness in America a remarkable addition to the ranks of the investigators of animal morphology.

The vast extent of territory over which our students are and will be scattered renders it difficult for many to keep in touch with the scientific advances which are being made in distant parts of the country, a necessary condition for effi-

cient and profitable work, where specialization is an inevitable concomitant of successful investigation.

It has been considered advisable that some means should be devised whereby this scientific isolation, felt by most investigators, should be broken up, and it is proposed to form in connection and affiliation with the American Society of Naturalists an Association of Morphologists, which shall hold stated meetings during the Christmas vacation, at which special and general morphological problems may be brought forward and discussed.

The time of year chosen is one that recommends itself to most investigators as interfering least with their private and collegiate work, and it has been thought well for the Association to avail itself of the many advantages resulting from a connection with the American Society of Naturalists.

The undersigned, therefore, call a meeting of those interested in the formation of an Association of American Morphologists, to be held in Boston (the details of the meeting to be announced later) on Monday, December 29, 1890, and respectfully request your coöperation.

If the formation of such a Society meets with your approval, and you desire to assist in its formation, please notify Dr. J. Playfair McMurrich, Clark University, Worcester, Mass., to that effect, in order that notices of further arrangements for the meetings may be sent to you.

(Signed) C. O. WHITMAN,
HENRY F. OSBORN,
E. B. WILSON,
EDWARD G. GARDINER,
J. PLAYFAIR McMURRICH.

The first meeting was held December 29, 1890, at the Massachusetts Institute of Technology, Dr. E. G. Gardiner being called to the chair and Dr. J. Playfair McMurrich acting as Secretary. Organization followed, after which Dr. E. B. Wilson was elected Chairman and Dr. J. Playfair McMurrich Secretary-Treasurer for the meeting.

The original members with their addresses at the time of this meeting, are given in the following list:

(The list is copied from the minutes of the first meeting.)

AYERS, HOWARD, Ph.D., The Lake Laboratory, Milwaukee, Wis.
ANDREWS, E. A., Ph.D., Johns Hopkins University, Baltimore, Md.
BAUR, G., Ph.D., Clark University, Worcester, Mass.
BUMPUS, H. C., Ph.B., Brown University, Providence, R. I.
CLARKE, S. F., Ph.D., Williams College, Williamstown, Mass.
COPE, E. D., 2102 Pine Street, Philadelphia, Pa.
GARDINER, E. G., Ph.D., Mass. Inst. of Technology, Boston, Mass.
HYATT, ALPHEUS, Boston Society of Natural History, Boston, Mass.
MALL, F. P., M.D., Clark University, Worcester, Mass.
MARK, E. L., Ph.D., Harvard College, Cambridge, Mass.
McMURRICH, J. P., Ph.D., Clark University, Worcester, Mass.
MINOT, C. S., D.Sc., Harvard Medical School, Boston, Mass.
MORSE, E. S., Ph.D., Peabody Academy of Science, Salem, Mass.

MORGAN, T. H., Ph.D., Johns Hopkins University, Baltimore, Md.
 OSBORN, H. F., D.Sc., Princeton College, Princeton, N. J.
 PARKER, G. H., B.Sc., Harvard College, Cambridge, Mass.
 RANKIN, W. M., Ph.D., Princeton College, Princeton, N. J.
 SCOTT, W. B., Ph.D., Princeton College, Princeton, N. J.
 SCUDDER, S. H., Cambridge, Mass.
 SMITH, S. I., Ph.D., Yale University, New Haven, Conn.
 WATASE, S., Ph.D., Clark University, Worcester, Mass.
 WHEELER, W. M., Clark University, Worcester, Mass.
 WHITMAN, C. O., Ph.D., Clark University, Worcester, Mass.
 WILSON, E. B., Ph.D., Bryn Mawr College, Bryn Mawr, Pa.
 WILSON, H. V., Ph.D., United States Fish Commission, Woods Hole, Mass.
 WRIGHT, R. R., B.Sc., University of Toronto, Canada.

CENTRAL NATURALISTS

The Central Naturalists first met in response to the following call:

CHICAGO, December 8, 1899.

DEAR SIR—The desirability of forming a Western branch of the American Society of Naturalists, with the same objects and conditions of membership as the main society, has long been under consideration by the Naturalists of the Central and Western States.

For the purpose of starting such a branch if it seems, on discussion, desirable (the main society acquiescing), this call is issued for a meeting of members of the American Society of Naturalists and affiliated scientific societies living west of the Alleghanies and of others interested in providing for an annual meeting of the Western Naturalists; the present meeting to be held at the Hull Biological Laboratories, University of Chicago, Thursday and Friday, December 28 and 29, 1899.

The provisional program is as follows:

Thursday, 10 a.m.—General meeting in Room 23, Botany Building, for organization and reading of papers. 1-2 p.m., Luncheon at Quadrangle Club. 3 p.m., Discussion: Methods and Results of Limnological Work. 6.30 p.m., Dinner at the Quadrangle Club.

Friday, 9 a.m.—General meeting for reading of papers.

You are respectfully invited to be present at the meeting. Titles of papers to be read should be sent to C. B. Davenport, 5725 Monroe avenue, Chicago, so as to reach him before December 16, at which time a second circular will be sent out. A few persons can probably be accommodated in the dormitories of the University without charge. Such rooms will be assigned to earliest applicants. Please reply to this letter before December 15.

C. R. BARNES,
 H. H. DONALDSON,
 S. A. FORBES,
 WILLIAM A. LOCY.
 JACOB REIGHARD.

(Among other Naturalists who have signified their approval of this call are: Prof. C. L. Edwards, University of Cincinnati; Prof. Carl Eigenmann, University of Indiana; Dr. H. S. Jennings, University of Michigan; Prof. H. V. Neal, Knox College, Illinois; Dr. W. S. Nickerson, University of Minnesota; Prof. C. P. Sigerfoos, University of Minnesota; Prof. H. B. Ward, University of Nebraska, and Prof. C. O. Whitman, University of Chicago.)

The following year a notice was issued as follows:

CHICAGO, ILL., November 15, 1900.

DEAR SIR—The meeting of Naturalists of the Central and Western States at Chicago last year (see *Science* for February 16, 1900) was so successful that a second meeting will be held at the Hull Biological Laboratories, University of Chicago, Thursday and Friday, December 27 and 28, 1900. It is expected that a permanent organization will be effected at this meeting.

The provisional program is as follows:

Thursday, 10 a.m.—General meeting in Room 24, Zoölogical Building (furnished with a projecting lantern), for organization and reading of the more general papers. 1 to 2 p.m., Luncheon at the Quadrangle Club. 3 p.m., Discussion: State Natural History Surveys; Methods, results, coöperation. 6.30 p.m., Dinner at the Quadrangle Club.

Friday, 8 a.m.—General meeting for reading of papers. At this time at least two sections, one in Zoölogy and one in Botany, will be formed, at which the more special papers will be read.

You are respectfully invited to be present at the meeting and to bring your advanced students. Titles of papers to be read should be sent to C. B. Davenport, 5725 Monroe Avenue, Chicago, or to C. R. Barnes, University of Chicago. A second circular will be sent to all who attended last year's meeting and to those who request it in reply to this letter before December 14.

* * * * *

Committee on Meeting:

E. A. BIRGE, *Chairman*,
C. R. BARNES,
T. G. LEE,
C. C. NUTTING,
C. B. DAVENPORT, *Secretary*.

The attendance at the second meeting exceeded 100 and it became necessary to hold two sections, a zoölogical and a botanical. This division paved the way for the formation, the following year, of a distinct zoölogical society.

ZOÖLOGICAL SOCIETY OF AMERICA

In December, 1901, the Eastern and Central Naturalists met together at Chicago and all the Zoölogists met with the American Morphological Society. In accordance with a desire that had been expressed among the "Central Naturalists," the Zoölogists and Botanists of that body met separately during one of the days and organized. The Zoölogists met at Kent Theatre, University of Chicago, on January 1, 1902, in response to a call. Dr. C. B. Davenport was

chosen to preside. A committee of three, consisting of Professors Reighard, Eigenmann and Davenport, was appointed to draft a constitution to be acted on at the next meeting and the body then adjourned to meet in Washington the following year. At a meeting of the Committee on Organization a constitution was drawn up, the name "Zoölogical Society of America" being selected.

CENTRAL AND EASTERN BRANCHES OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

In Convocation week, 1902-03, the Zoölogists of the Central and Western States met in Washington with their Eastern brethren. At this time they adopted the constitution drawn up by the joint constitutional committee appointed at the joint Washington meeting. By this and further appropriate action they organized as the Central Branch of the American Society of Zoölogists, the American Morphological Society becoming at the same time the Eastern Branch of the same society.

AMERICAN SOCIETY OF ZOÖLOGISTS

The present organization of the American Society of Zoölogists, effected at a joint meeting of the Eastern and Central Branches of the Society at Philadelphia in 1913, grew out of a general feeling of dissatisfaction with provisions of the constitution relating to the holding of joint meetings of the two Branches and the election of members.

At the annual meeting of the Eastern Branch of the Society in Ithaca in 1910 the Executive Committee was instructed to consider the advisability of changes in the constitution with reference to these matters. The following quotation is taken from the report of this Committee to the Society at a joint meeting held at Princeton in 1911:

"At the Ithaca meeting of the Society it was voted that the Executive Committee be instructed to consider the advisability of changes in the constitution with reference to the place of meeting and the election of members. Acting under these instructions your Committee has carefully considered the points raised in the motion quoted and certain related matters as well. Our conclusions are as follows:

"1. That the arrangements at present existing between the Eastern and Central Branches of the Society in respect to (a) the holding of joint meetings, (b) the election of members, and (c) other minor administrative matters, have not been found to be satisfactory in practical operation. The feeling of dissatisfaction regarding existing conditions is not confined to the membership of either Branch, but is general throughout the Society. On this account steps should be taken at once to bring about, if possible, a more satisfactory scheme of organization and administration of the Society.

"2. That, in the opinion of the Committee, there are essentially but two general plans of action which offer any hope of a practical solution of the difficulties. These are:

"(a) The plan of *amalgamation* of the Branches into one single unitary organization. This would mean the abolition of Eastern and Central Branches as such, the election of a single set of officers for the Society as a whole, and the holding of but one meeting annually. The place and time of this meeting could

be decided by the Executive Committee each year independently on the basis of the merits of the whole situation as it appears at the time. Should this plan be adopted, however, it would, in our judgment, be absolutely essential that some provision be made whereby the holding of a fair proportion of the meetings of the Society in central territory would be assured.

"(b) The plan of *separation* of the Branches into two distinct and independent societies, each having its own officers, and meeting whenever and wherever it pleased. Should such a plan prevail, however, it would appear to be highly desirable that nothing should be put into the constitution or by-laws of either society which would in any way hinder or prevent the holding of a joint meeting of the two societies, whenever such course seemed desirable.

"Feeling that the problem before us is one of great importance, upon a wise and just solution of which the future welfare and dignity of the Society must in some measure depend, your Committee would recommend:

"1. The appointment at this meeting of a Committee on Organization and Policy to consist of three members of the Eastern Branch and three members of the Central Branch and the President of the Society *ex officio*, to consider during the ensuing year the problem of the organization of the Society and to prepare a new constitution and a set of by-laws."

The Committee on Organization and Policy, appointed at the Princeton meeting, is as follows:

E. G. CONKLIN,	}	Representing the Eastern Branch.
G. A. DREW,		
R. G. HARRISON,		
F. R. LILLIE,	}	Representing the Central Branch.
M. M. METCALF,		
W. A. LOCY,		

This Committee made no report at the joint meeting held at Cleveland in 1912, but during the summer of 1913 a meeting of the Committee, called by the president of the Society, H. B. Ward, was held at Woods Hole, at which a constitution for the Society was outlined. At this meeting the absent members, H. V. Wilson, M. M. Metcalf and W. A. Locy, were represented by G. H. Parker, George Lefevre and Jacob Reighard. The draft of the constitution formulated at this meeting was later submitted by the chairman of the Committee, G. A. Drew, to all members of the original committee, and the constitution finally agreed upon is practically the same as that adopted by the Society at the joint meeting held at Philadelphia in 1913 (see page 71).

LIST OF FORMER OFFICERS

AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson	J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. M. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

AMERICAN SOCIETY OF ZOOLOGISTS

<i>EASTERN BRANCH</i>	<i>President</i>	<i>CENTRAL BRANCH</i>
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward
Jacob Reighard	1903	H. F. Nachtrieb
W. E. Castle	1904	S. J. Holmes
William Patten	1905	William A. Locy
William Patten	1906	George Lefevre
F. H. Herrick	1907	H. B. Ward
H. S. Jennings	1908	M. F. Guyer
H. V. Wilson	1909	M. F. Guyer

EASTERN BRANCH	<i>Vice-President</i>	CENTRAL BRANCH
H. H. Wilder	1910	H. F. Nachtrieb
H. E. Crampton	1911	R. H. Walcott
G. A. Drew	1912	C. M. Child
Alex. Petrunkevitch	1913	C. M. Child

Secretary-Treasurer

G. A. Drew	1903	Frank Smith
G. A. Drew	1904	F. R. Lillie
H. S. Pratt	1905	C. E. McClung
H. S. Pratt	1906	T. G. Lee
C. J. Herrick	1907	T. G. Lee
L. L. Woodruff	1908	T. G. Lee
L. L. Woodruff	1909	Charles Zeleny
H. W. Rand	1910	H. V. Neal
Raymond Pearl	1911	H. V. Neal
J. H. Gerould	1912	W. C. Curtis
Caswell Grave	1913	W. C. Curtis

Executive Committeemen

EASTERN BRANCH	CENTRAL BRANCH
F. R. Lillie	George Lefevre
T. H. Montgomery	T. G. Lee
H. C. Bumpus	Herbert Osborn
H. S. Jennings	C. H. Eigenmann
E. A. Andrews	J. G. Needham
W. R. Coe	S. J. Holmes
G. A. Drew	W. A. Loey
M. M. Metcalf	C. M. Child
D. H. Tennent	R. H. Walcott
R. G. Harrison	W. C. Curtis
H. E. Jordan	Oscar Riddle
C. E. McClung	H. B. Ward
	Chauncey Juday
	H. W. Norris
	C. E. McClung
	H. F. Nachtrieb

AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914. C. E. McClung	M. F. Guyer	H. E. Jordan
1915. W. A. Loey	W. E. Ritter	H. F. Nachtrieb
1916. D. H. Tennent	Charles Zeleny	H. V. Wilson

LIST OF PLACES OF MEETING

AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago	1900—Chicago
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SOCIETY OF AMERICAN ZOÖLOGISTS

1901—Chicago	1902—Washington
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AMERICAN SOCIETY OF ZOÖLOGISTS

EASTERN BRANCH	JOINT MEETINGS	CENTRAL BRANCH
1903—Philadelphia	1905—Ann Arbor	1903—St. Louis
1904—Philadelphia	1908—Baltimore	1905—(Mch.) Chicago
1906—New York	1911—Princeton	1907—(Mch.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana

AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

1914. Philadelphia	1915. Columbus
1916. New York	

AMERICAN SOCIETY OF ZOÖLOGISTS

OFFICERS AND LIST OF MEMBERS OF THE SOCIETY

Officers

<i>President</i>	MAYNARD MAYO METCALF
<i>Vice-President</i>	CHARLES ZELENY
<i>Secretary-Treasurer</i>	CASWELL GRAVE

Executive Committee

GEORGE LEFEVRE	L. J. COLE
A. F. SHULL	R. P. BIGELOW
H. V. WILSON	

HONORARY MEMBER

James Viscount Bryce, Hindleap, Forest Row, Sussex, England.

MEMBERS

- ABBOTT, JAMES FRANCIS, A.B., A.M. (Leland Stanford), Ph.D. (Chicago), Professor of Zoölogy, *Washington University, St. Louis, Mo.*
- ACKERT, JAMES EDWARD, A.B., A.M., Ph.D. (University of Illinois), Assistant Professor of Zoölogy, *Kansas State Agricultural College, Manhattan, Kan.*
- ALLEE, WARDER CLYDE, S.B. (Earlham College), S.M. (Chicago), Ph.D. (Chicago), Professor of Biology, *Lake Forest College, Lake Forest, Ill.*
- ALLEN, BENNET MILLS, Ph.B. (De Pauw), Ph.D. (Chicago), Professor of Zoölogy, *University of Kansas, Lawrence, Kan.*
- ALLEN, EZRA, A.M., Ph.D. (University of Pennsylvania), Professor of Biology, *Philadelphia School of Pedagogy, 12th above Spring Garden, Philadelphia, Pa.*
- ANDREWS, ETHAN ALLEN, Ph.B. (Yale), Ph.D. (Johns Hopkins), Life Member. Professor of Zoölogy, *Johns Hopkins University, Baltimore, Md.*
- AREY, LESLIE BRAINERD, Ph.D. (Harvard), Instructor in Anatomy, *Northwestern University Medical School, 2431 Dearborn Street, Chicago, Ill.*
- BAITSELL, GEORGE ALFRED, B.S. (Central College, Iowa), M.A., Ph.D. (Yale), Instructor in Biology in Yale University, *Osborn Zoölogical Laboratory, Yale Station, New Haven, Conn.*
- BANTA, ARTHUR MANGUN, A.B., A.M. (Indiana), Ph. D. (Harvard), Resident Investigator, *Station for Experimental Evolution, Carnegie Institution, Cold Spring Harbor, Long Island, N. Y.*
- BARDEEN, CHARLES RUSSELL, A.B. (Harvard), M.D. (Johns Hopkins), Professor of Anatomy and Dean of the College of Medicine, *University of Wisconsin, Madison, Wis.*

- BARKER, FRANKLIN D., A.B., A.M. (Ottawa University, Kansas), Ph.D. (Nebraska), Professor of Medical Zoölogy and Parasitology, *University of Nebraska, Station A, Lincoln, Neb.*
- BARROWS, WILLIAM MARTIN, B.S. (Michigan Agricultural College), S.B., S.M. (in Biology) (Harvard), *371 Harvard Street, Cambridge, Mass.*
- BARTELMIZ, GEORGE W., Ph.D. (Chicago), Assistant Professor of Anatomy, *University of Chicago, Chicago, Ill.*
- BASSET, GARDNER CHENEY, Ph.D. (Johns Hopkins), *University of Pittsburgh, Pittsburgh, Pa.*
- BAUMGARTNER, WILLIAM JACOB, A.B., A.M. (Kansas), Associate Professor of Zoölogy, *University of Kansas, 1209 Ohio Street, Lawrence, Kan.*
- BECKWITH, CORA JIPSON, B.S. (Michigan), M.A., Ph.D. (Columbia), Associate Professor of Zoölogy, *Vassar College, Poughkeepsie, N. Y.*
- BIGELOW, MAURICE ALPHEUS, B.S. (Ohio Wesleyan), M.S. (Northwestern), Ph.D. (Harvard), Professor of Biology, *Teachers' College, 525 West 120th Street, New York City.*
- BIGELOW, ROBERT PAYNE, S.B. (Harvard), Ph.D. (Johns Hopkins), Associate Professor of Zoölogy and Parasitology, *Massachusetts Institute of Technology, Cambridge, Mass.*
- BINFORD, RAYMOND, B.S. (Earlham), S.M. (Chicago), Ph.D. (Johns Hopkins), Professor of Zoölogy at Earlham College, *Earlham College, Richmond, Ind., Earlham P. O.*
- BLACKMAN, MAULSBY WILLETT, A.B., A.M. (Kansas), Ph.D. (Harvard), Professor of Forest Entomology and Head of Department of Forest Zoölogy, *New York State College of Forestry, at Syracuse University, 216 Stratford Street, Syracuse, N. Y.*
- BORING, ALICE MIDDLETON, A.B., A.M., Ph.D. (Bryn Mawr), Associate Professor of Zoölogy, *University of Maine, Orono, Maine.*
- BRUNER, HENRY LANE, A.B. (Abingdon), Ph.D. (Freiburg), Professor of Biology, *Butler College, 324 South Ritter Avenue, Indianapolis, Ind.*
- BUDINGTON, ROBERT ALLYN, B.A., M.A. (Williams), Professor of Zoölogy, *Oberlin College, Oberlin, Ohio.*
- BUMPUS, HERMON CAREY, Ph.D., LL.D. (Clark), Ph.B., D.Sc. (Brown), D.Sc. (Tufts) President of Tufts College, *Tufts College, Mass.*
- BURROWS, MONTROSE T., A.B. (Kansas), M.D. (Johns Hopkins), Acting Resident Pathologist, *Johns Hopkins Hospital, Baltimore, Md.*
- BYRNES, ESTHER F., Ph.D.,
193 Jefferson Avenue, Brooklyn, N. Y.
- CALKINS, GARY N., B.S. (Mass. Inst. Tech.), Ph.D., (Columbia) Professor of Protozoölogy, *Columbia University, New York City.*
- CALVERT, PHILIP POWELL, Ph.D. (Pennsylvania), Professor of Zoölogy, *University of Pennsylvania, Zoölogical Laboratory, Philadelphia, Pa.*
- CAROTHERS, E. ELEANOR, A.B., A.M., Ph.D., *Zoölogical Building, University of Pennsylvania, Philadelphia, Pa.*
- CARPENTER, FREDERIC WALTON, B.S. (New York Univ.), A.M., Ph.D., (Harvard), Professor of Biology, *Trinity College, Hartford, Conn.*
- CARY, LEWIS R., B.S., M.S. (Maine), M.A., Ph.D. (Princeton), Asst. Prof. of Biology, *Princeton University, Princeton, N. J.*

- CASTEEL, DANA BRACKENRIDGE, A.B. (Allegheny), A.M. (Ohio Wesleyan), Ph.D. (University of Pennsylvania), Associate Professor of Zoölogy, *University of Texas, Austin, Texas.*
- CASTLE, WILLIAM E., A.B. (Denison), A.M., Ph.D. (Harvard), Professor of Zoölogy in *Harvard University, Payson Road, Belmont, Mass.*
- CHAMBERLIN, RALPH V., B.S. (Utah), Ph.D. (Cornell), Curator of Arachnida, Myriopoda and Annelida, Mus. Comp. Zoölogy, *Harvard University, Museum Comp. Zoölogy, Cambridge, Mass.*
- CHAMBERS, ROBERT, JR., A.M. (Queen's Univ., Can.), Ph.D. (Munich), Instructor in Anatomy, *Cornell Medical College, 28th Street and First Avenue, New York City.*
- CHESTER, WAYLAND MORGAN, A.B., A.M., (Colgate Univ.), Professor of Biology, *Colgate University, Hamilton, N. Y.*
- CHILD, CHARLES MANNING, Ph.B., M.S. (Wesleyan), Ph.D. (Leipzig), Associate Professor of Zoölogy, *Hull Zoölogical Laboratory, University of Chicago, Chicago, Ill.*
- CHURCHILL, EDWARD PERRY, A.B. (Iowa), Ph.D. (Johns Hopkins), Assistant U. S. Bureau of Fisheries, *317 Marshall Street, Hampton, Va.*
- CLAPP, CORNELIA MARIA, Ph.B. (Syracuse), Ph.D. (Chicago), Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*
- CLARK, HOWARD WALTON, A.B., A.M. (Indiana), Scientific Assistant United States Bureau of Fisheries, *United States Biological Station, Fairport, Iowa.*
- COE, WESLEY R., Ph.D. (Yale), Professor of Biology, *Yale University, New Haven, Conn.*
- COGHILL, GEORGE E., A.B., Ph.D. (Brown), Professor of Anatomy, *University of Kansas, R. F. D. 9, Lawrence, Kan.*
- COLE, LEON J., A.B. (Michigan), Ph.D. (Harvard), Professor of Experimental Breeding, *College of Agriculture, University of Wisconsin, Madison, Wis.*
- COLTON, HAROLD SELLERS, B.S., M.A., Ph.D. (Pennsylvania), Assistant in Zoölogy, *University of Pennsylvania, Philadelphia, Pa.*
- CONGDON, EDGAR DAVIDSON, A.B., A.M. (Syracuse), Ph.D. (Harvard), *Leland Stanford Jr. University, 330 Coleridge Avenue, Palo Alto, Cal.*
- CONKLIN, EDWIN GRANT, B.S., A.B., A.M., Sc.D. (Hon.) (Ohio Wesleyan), Ph.D. (Johns Hopkins), Sc.D. (Hon.) (Pennsylvania), Professor of Biology, *Princeton University, Princeton, N. J.*
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